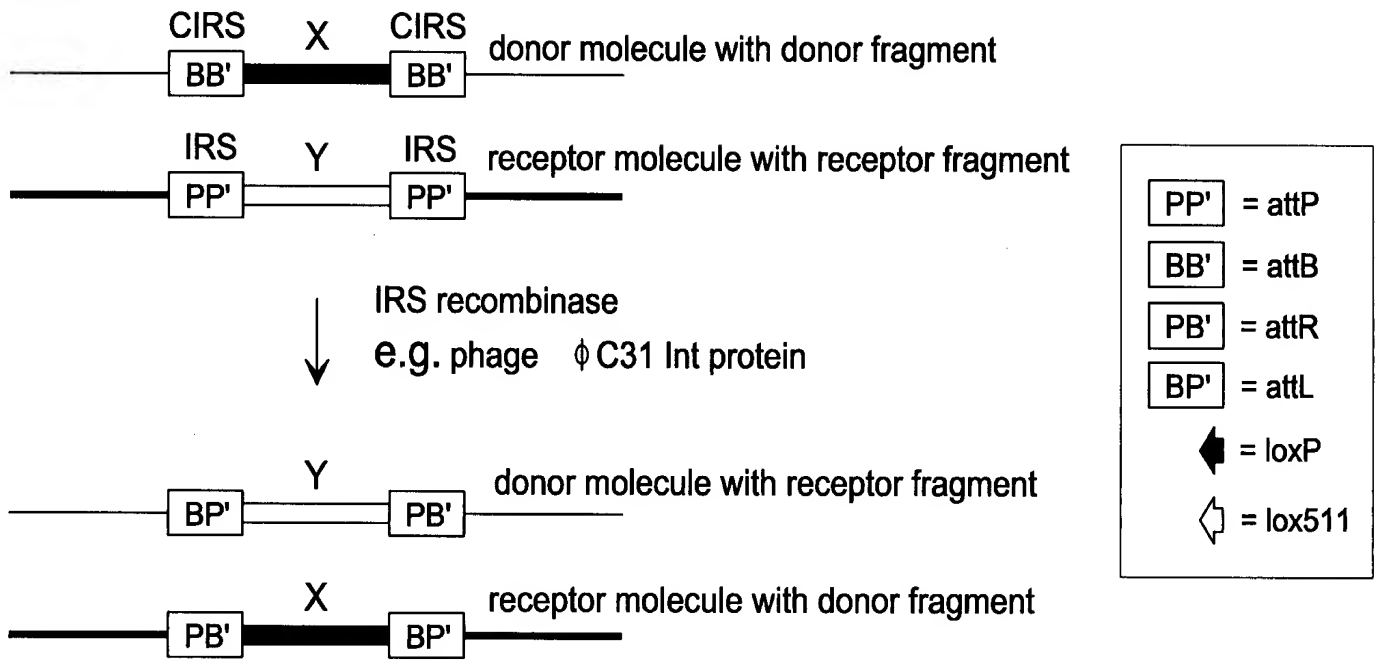


Figure 1A

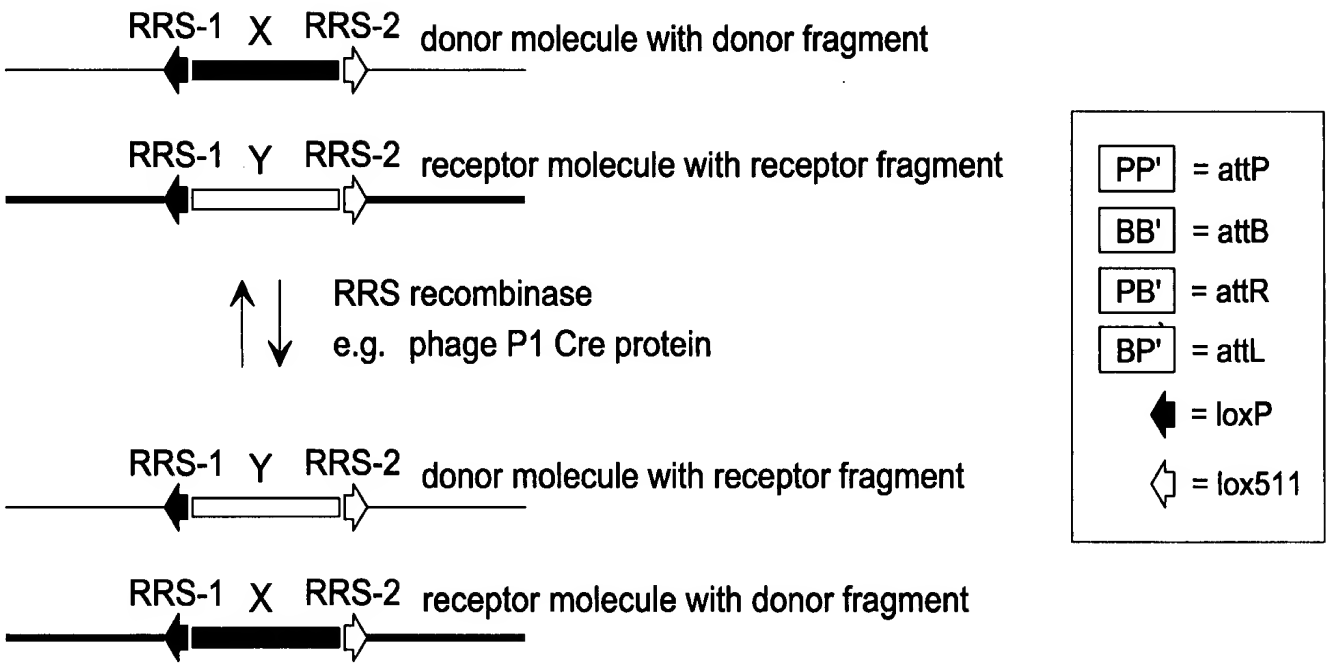

RRS-1 X RRS-2 donor molecule with donor fragment
RRS-1 Y RRS-2 receptor molecule with receptor fragment
RRS recombinase
e.g. phage P1 Cre protein
RRS-1 Y RRS-2 donor molecule with receptor fragment
RRS-1 X RRS-2 receptor molecule with donor fragment
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= loxP
= lox511

Figure 1B

Figure 2A

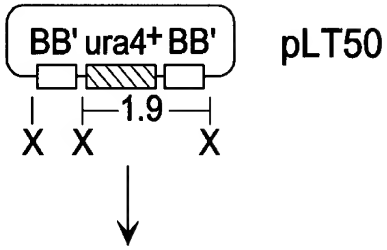


Figure 2B

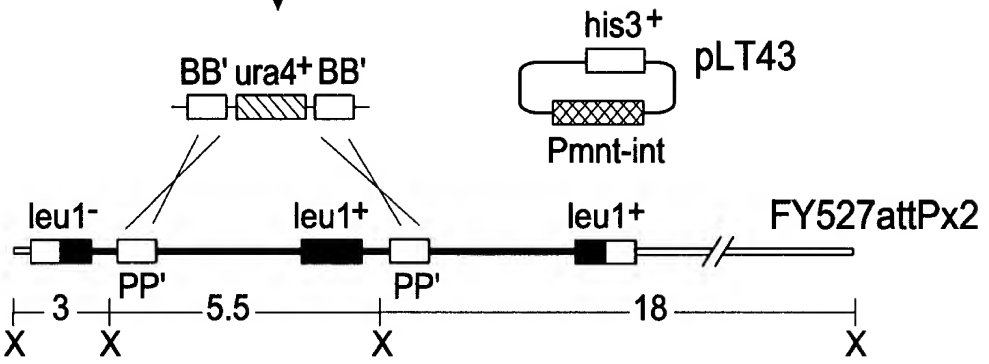


Figure 2C

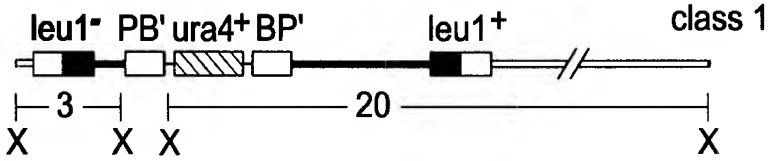


Figure 2D

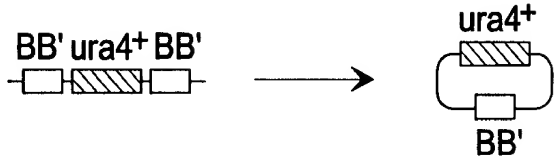


Figure 2E

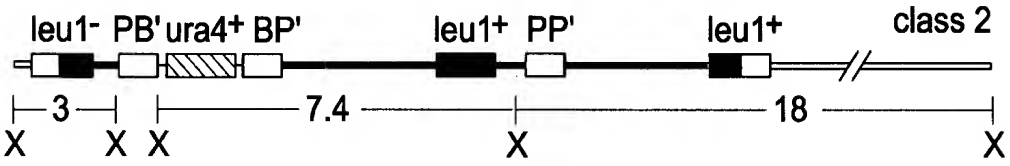


Figure 2F

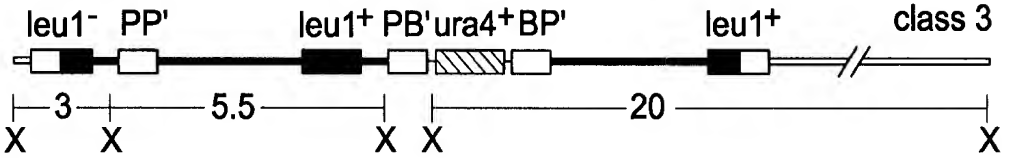
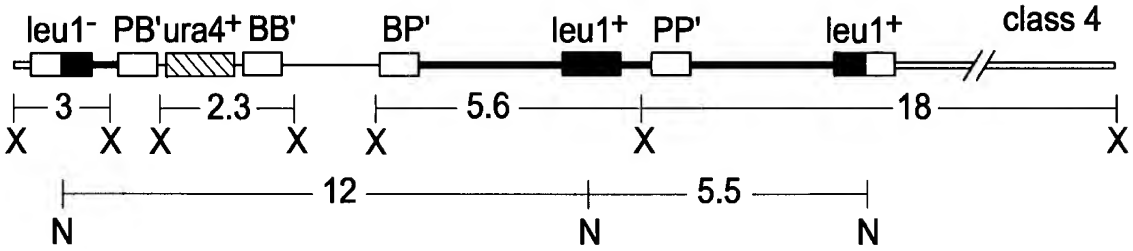


Figure 2G



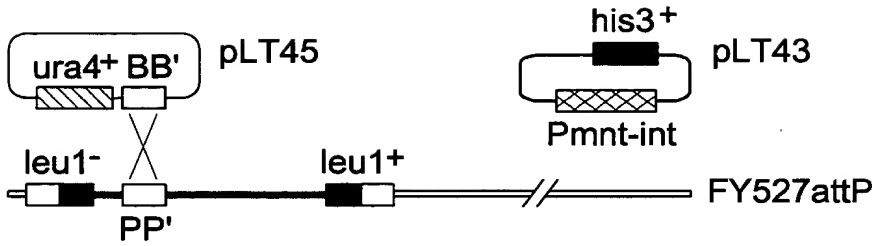


Figure 3A

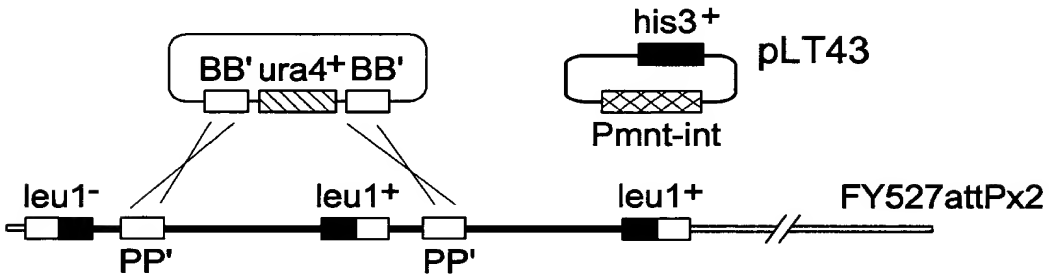


Figure 3B

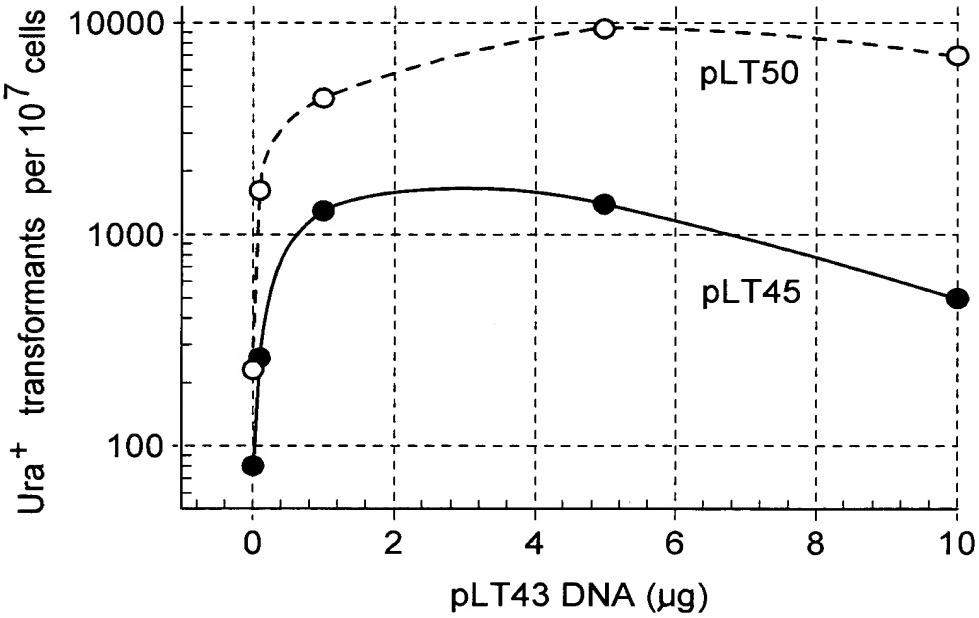


Figure 3C

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region
int = integrase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

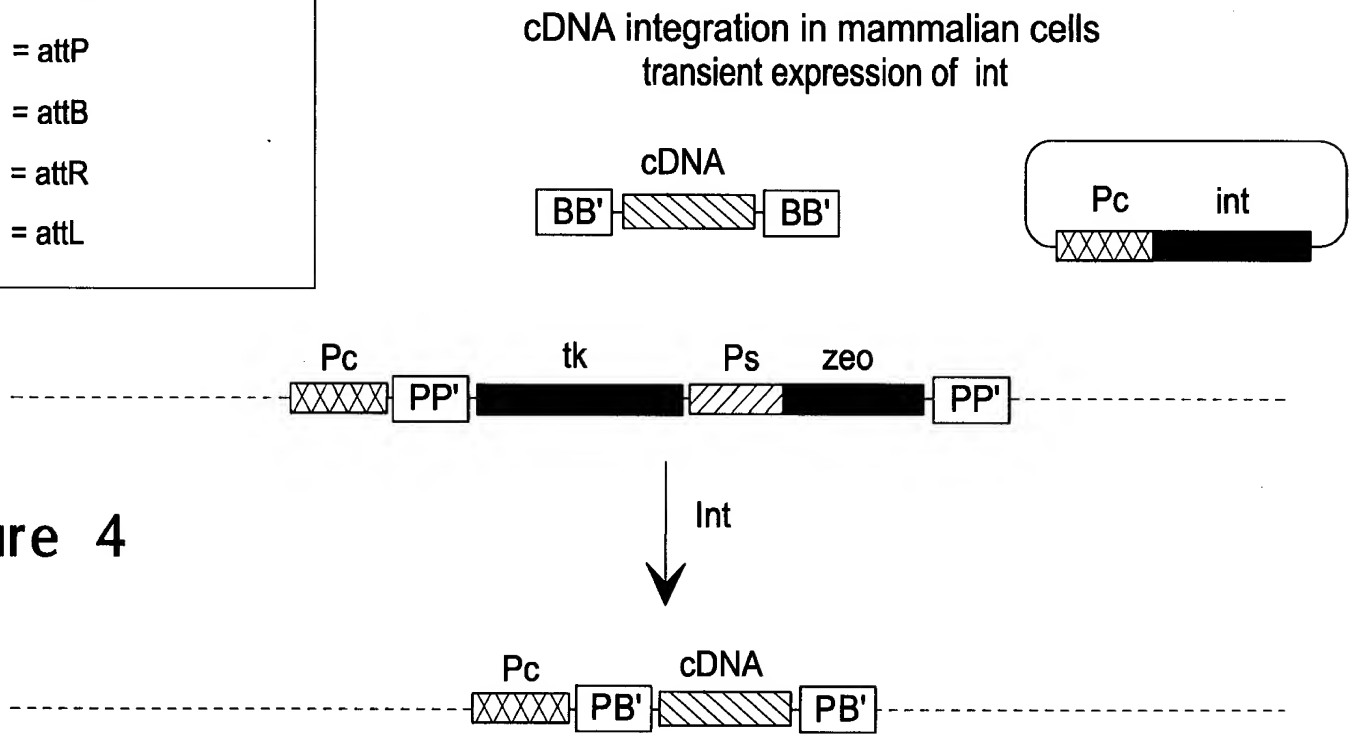


Figure 4

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

Strategy for cDNA integration in mammalian cells

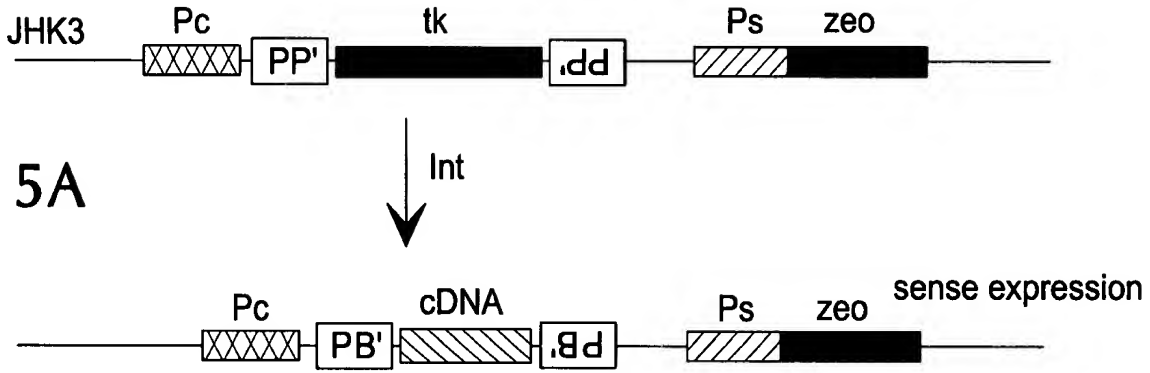


Figure 5A

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

Strategy for cDNA integration in mammalian cells

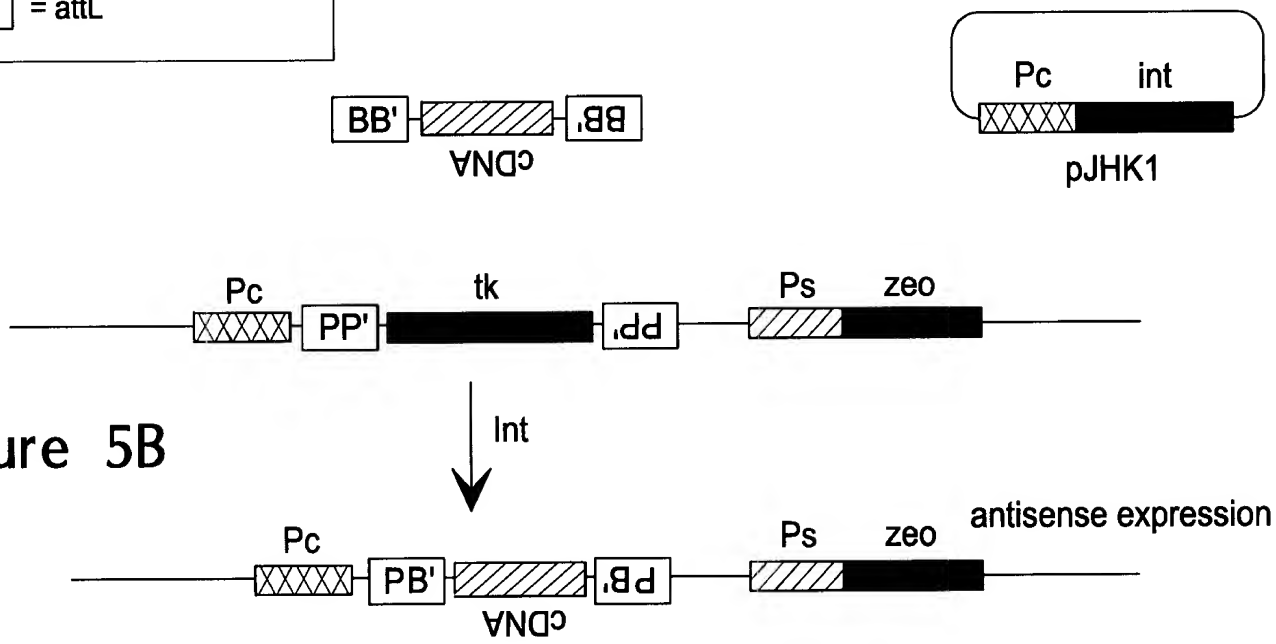
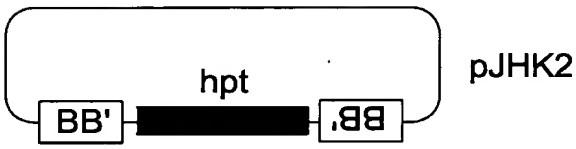


Figure 5B

Strategy for cDNA integration in mammalian cells



Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

Figure 5C

Strategy for cDNA integration in mammalian cells

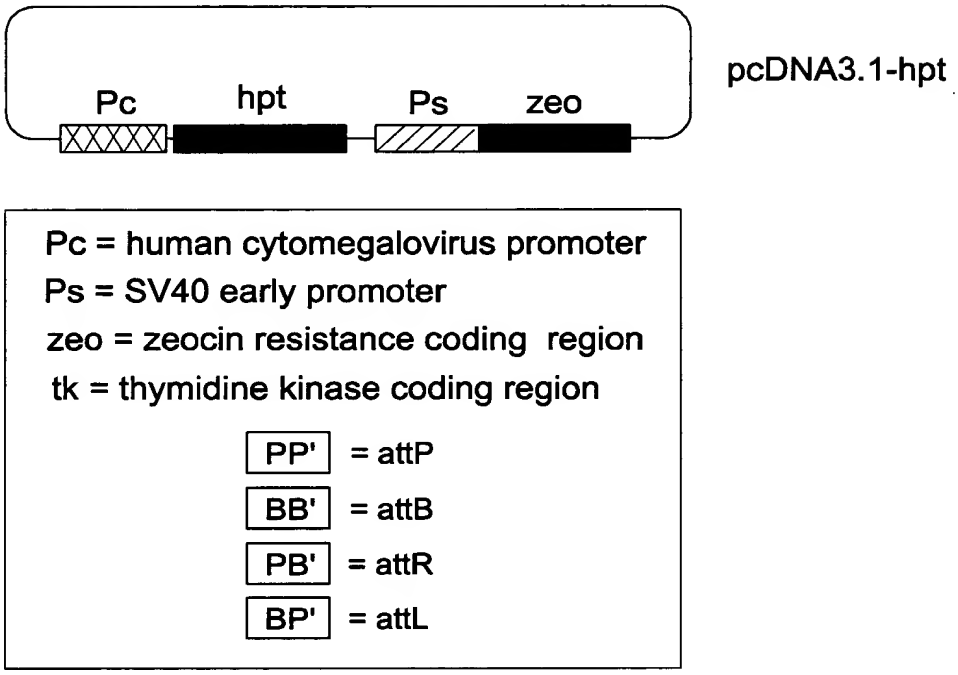


Figure 5D

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

Single copy target construct in human cells

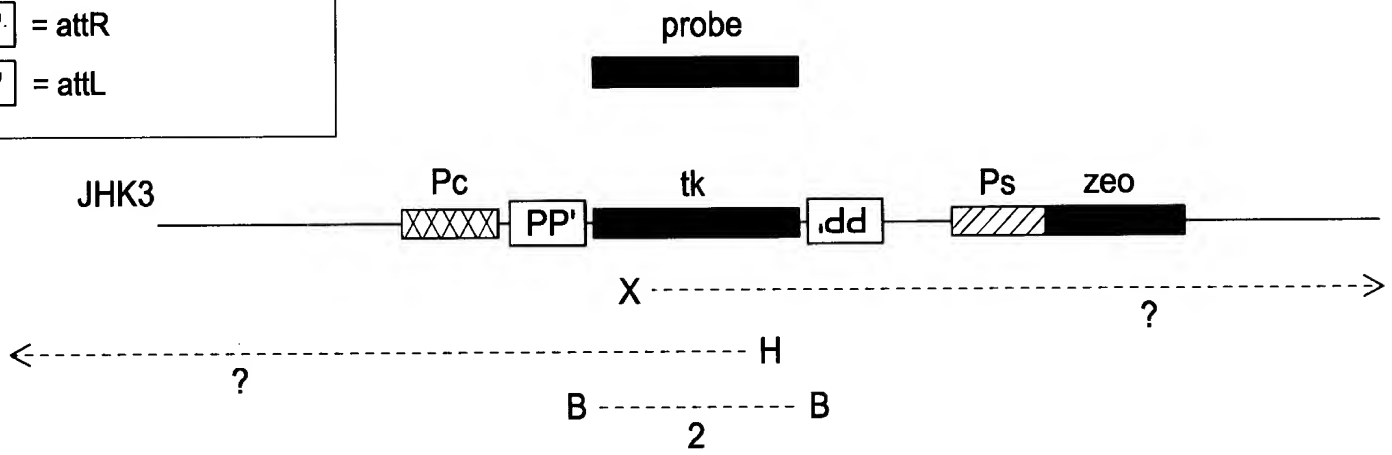


Figure 5E

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
PB' = attR
BP' = attL

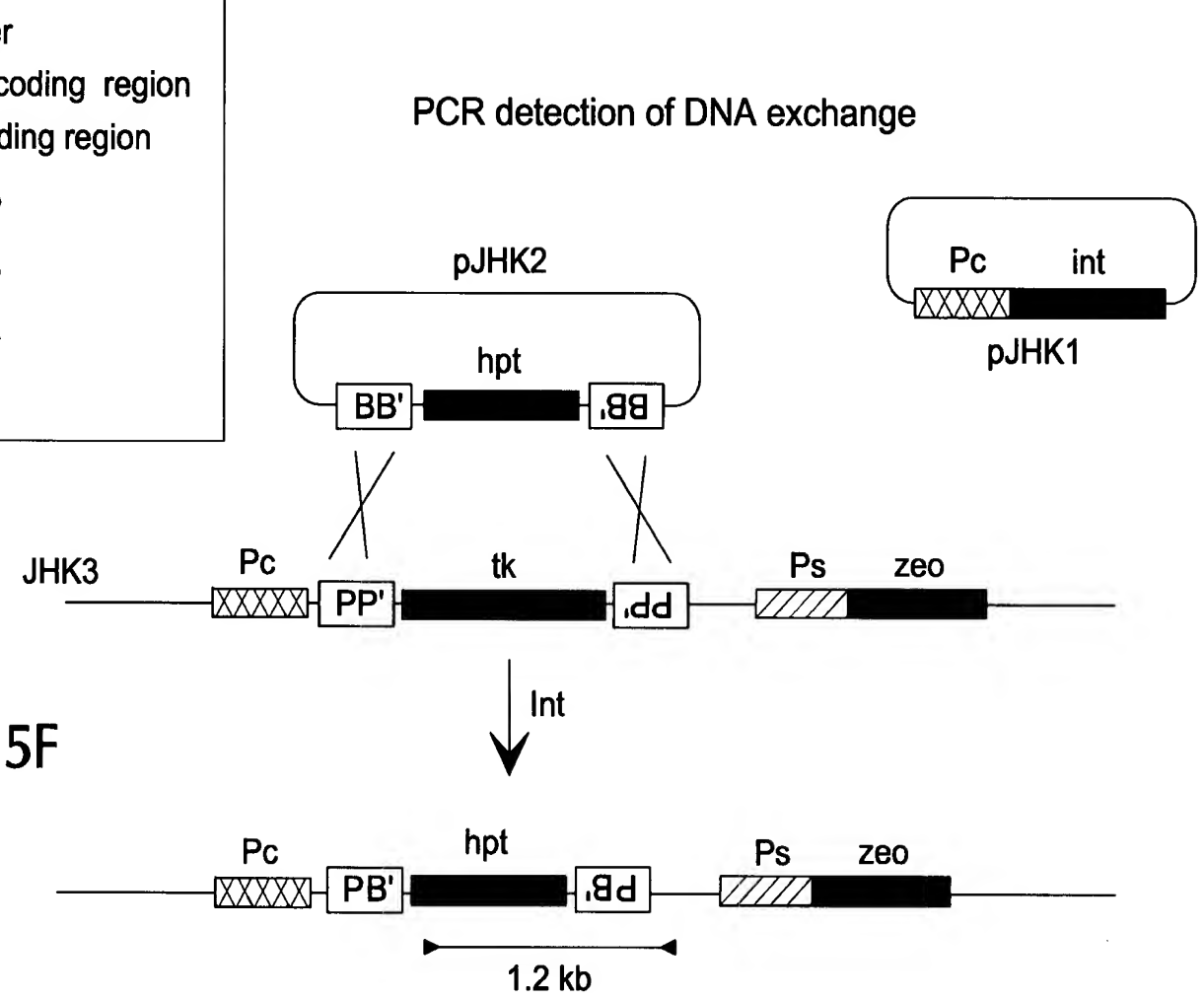


Figure 5F

P = promoter

35S = CaMV 35S promoter

npt = kanamycin resistance coding region

codA = cytosine deaminase coding region

int = integrase coding region

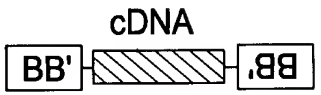
PP' = attP

BB' = attB

PB' = attR

BP' = attL

cDNA integration in plant cells
int expressed from target site



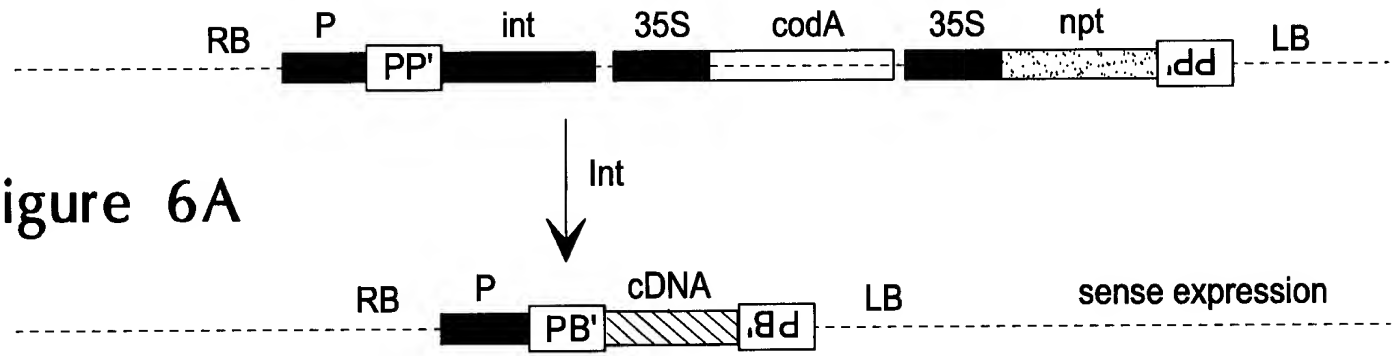


Figure 6A

P = promoter

35S = CaMV 35S promoter

npt = kanamycin resistance coding region

codA = cytosine deaminase coding region

int = integrase coding region

PP' = attP

BB' = attB

PB' = attR

BP' = attL

cDNA integration in plant cells
int expressed from target site

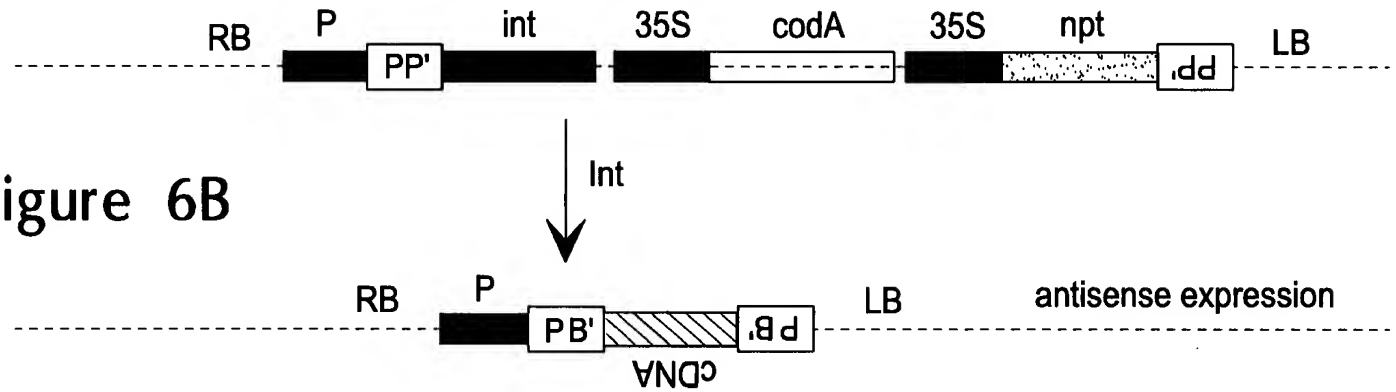


Figure 6B

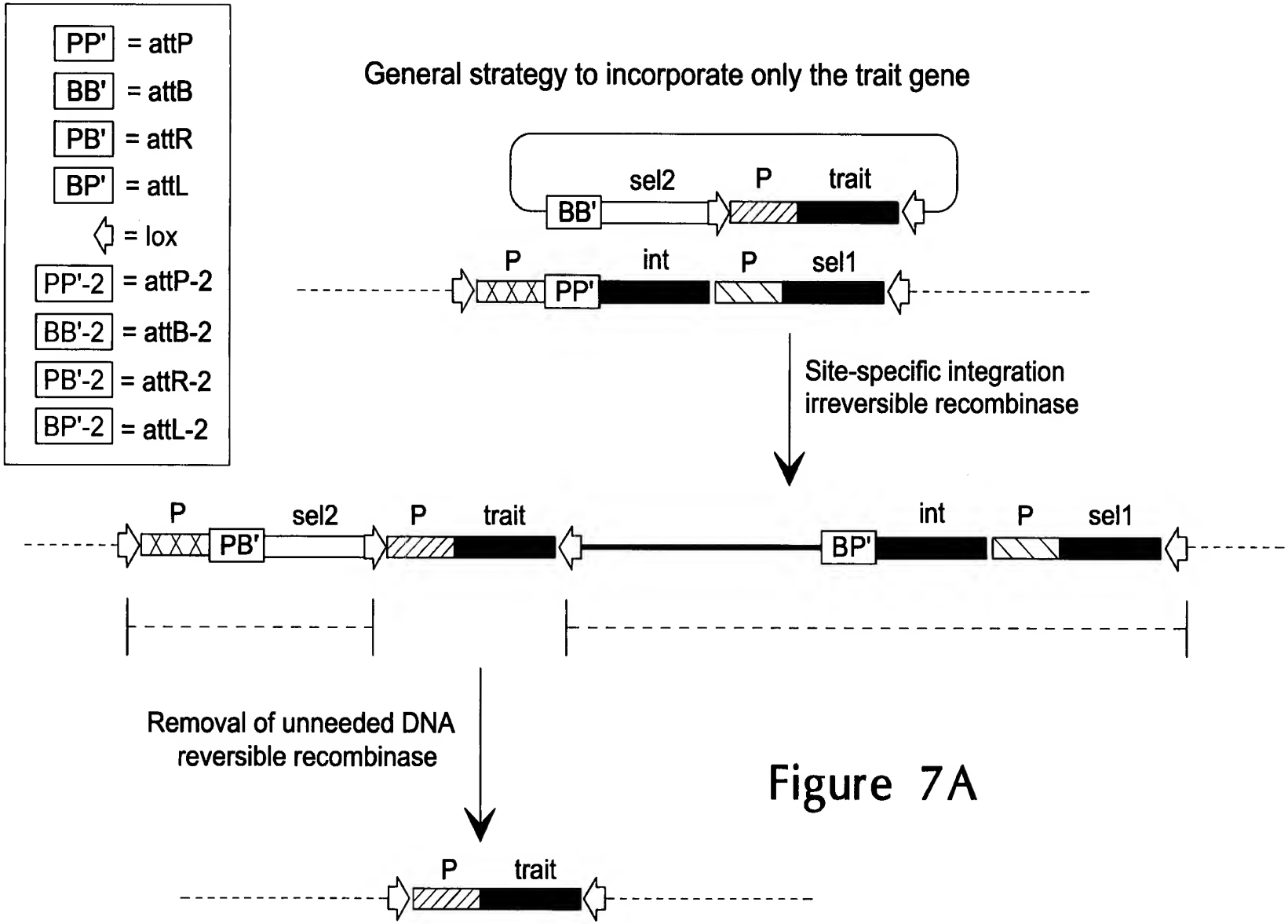

PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox
PP'-2 = attP-2
BB'-2 = attB-2
PB'-2 = attR-2
BP'-2 = attL-2
General strategy to incorporate only the trait gene
sel2
P
trait
BB'
P
int
P
sel1
PP'
Site-specific integration
irreversible recombinase
P
sel2
P
trait
int
P
sel1
PB'
BP'
Removal of unneeded DNA
reversible recombinase
P
trait

Figure 7A

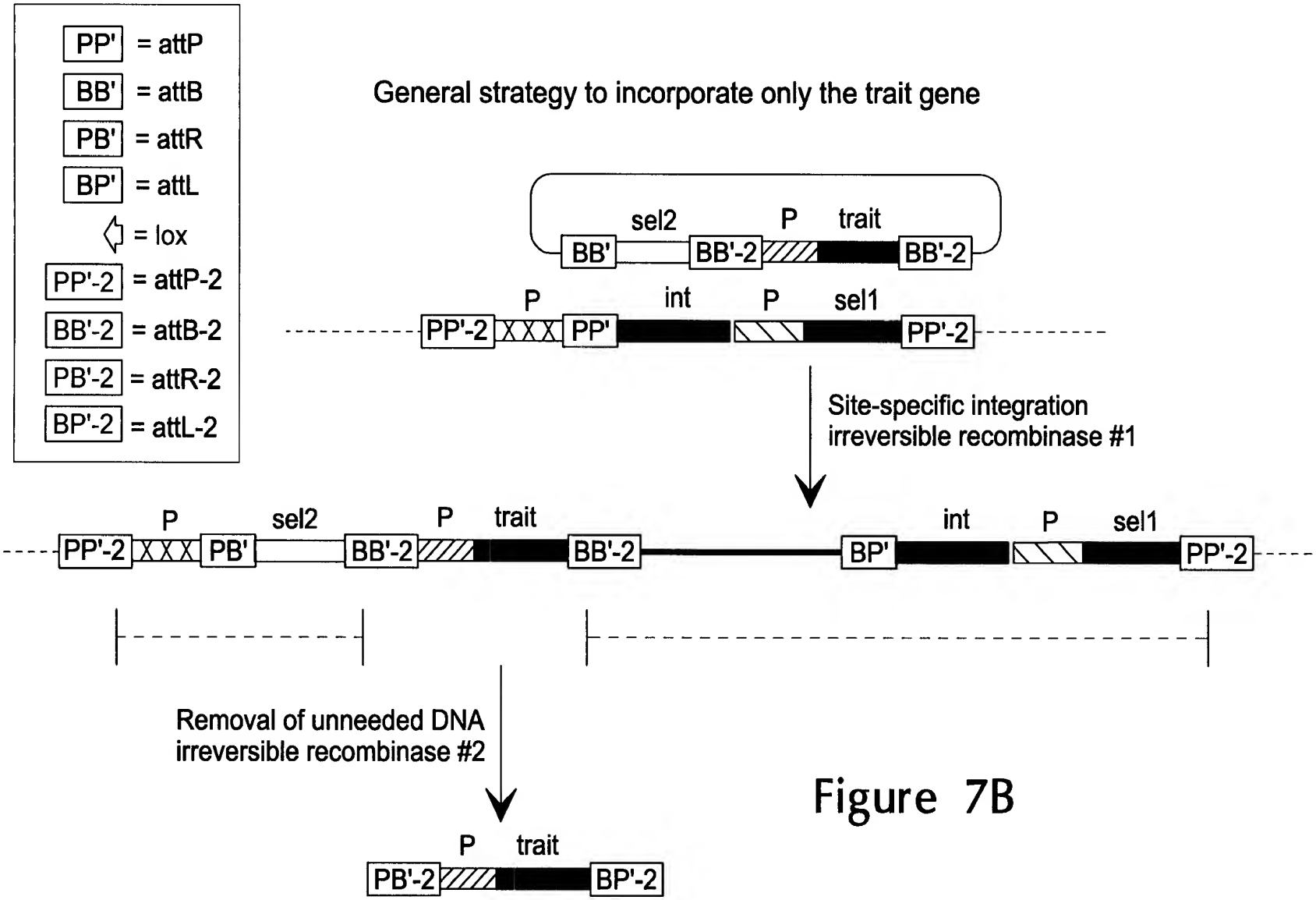

PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox
PP'-2 = attP-2
BB'-2 = attB-2
PB'-2 = attR-2
BP'-2 = attL-2
General strategy to incorporate only the trait gene
sel2
P
trait
BB'
BB'-2
BB'-2
P
int
P
sel1
PP'-2
PP'
PP'-2
Site-specific integration
irreversible recombinase #1
P
sel2
P
trait
int
P
sel1
PP'-2
PB'
BB'-2
BB'-2
BP'
PP'-2
Removal of unneeded DNA
irreversible recombinase #2
P
trait
PB'-2
BP'-2

Figure 7B

General strategy to stack genes, part1
Use of directly oriented sites

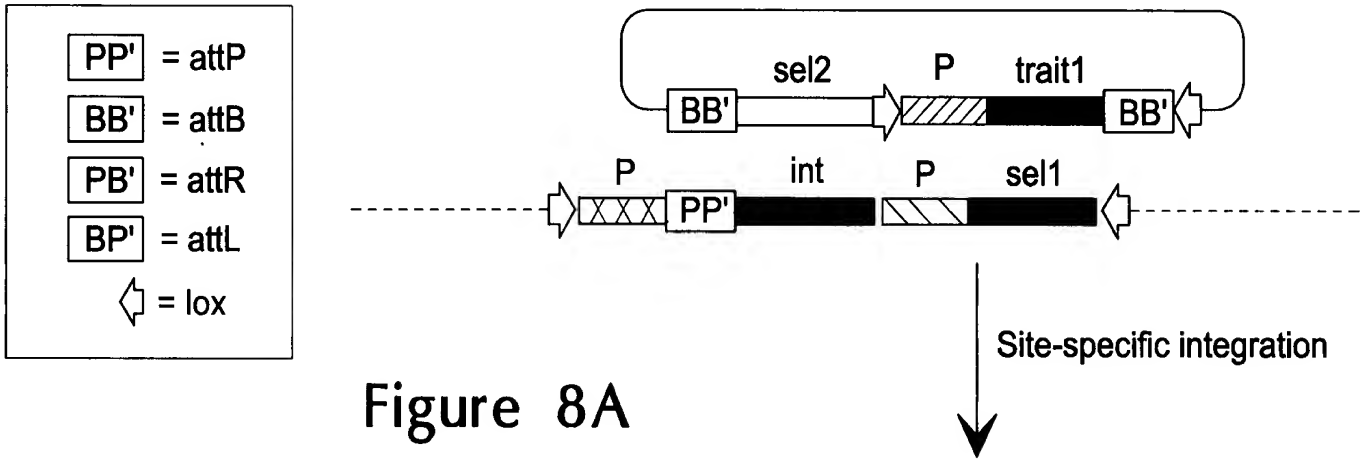


Figure 8A

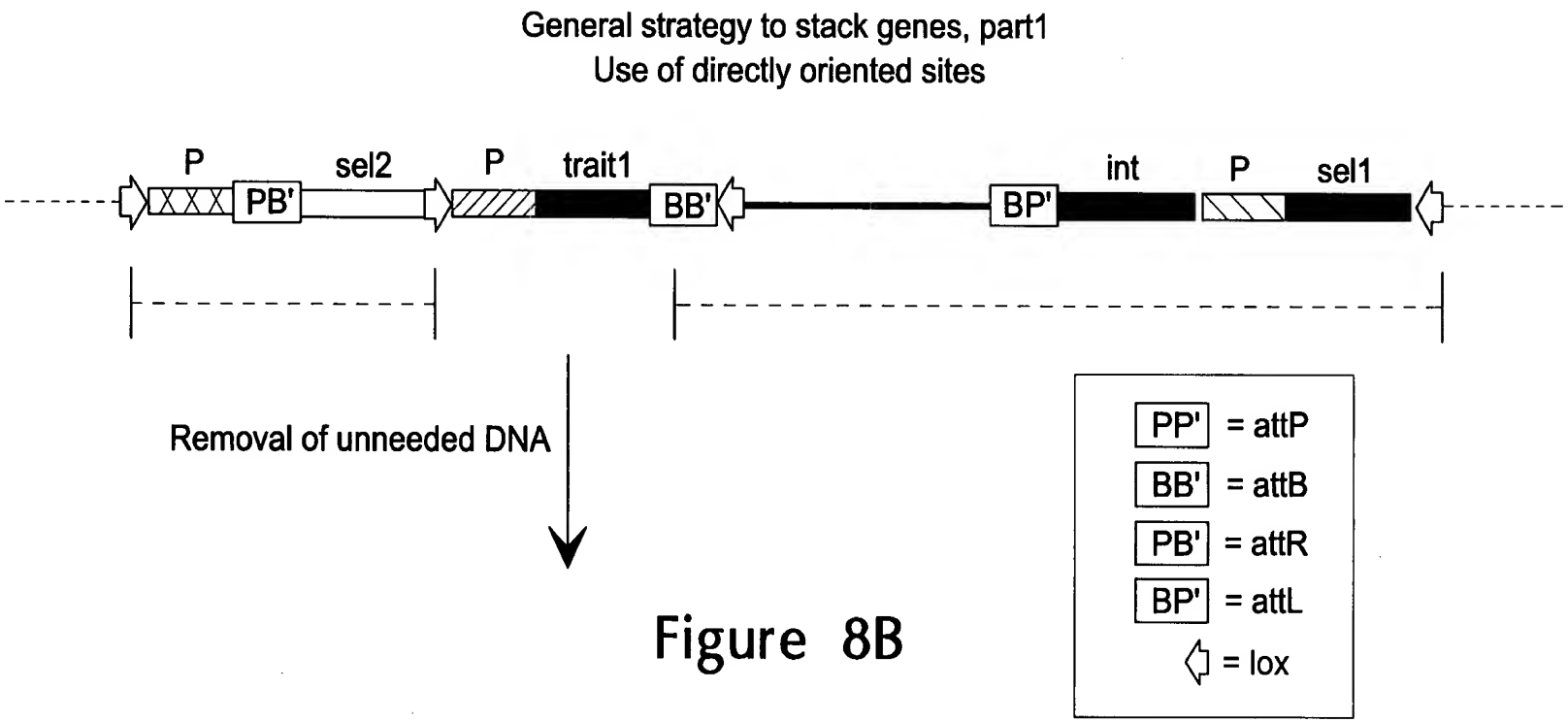

General strategy to stack genes, part1
Use of directly oriented sites
P
sel2
P
trait1
int
P
sel1
PB'
BB'
BP'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 8B

General strategy to stack genes, part1
Use of directly oriented sites

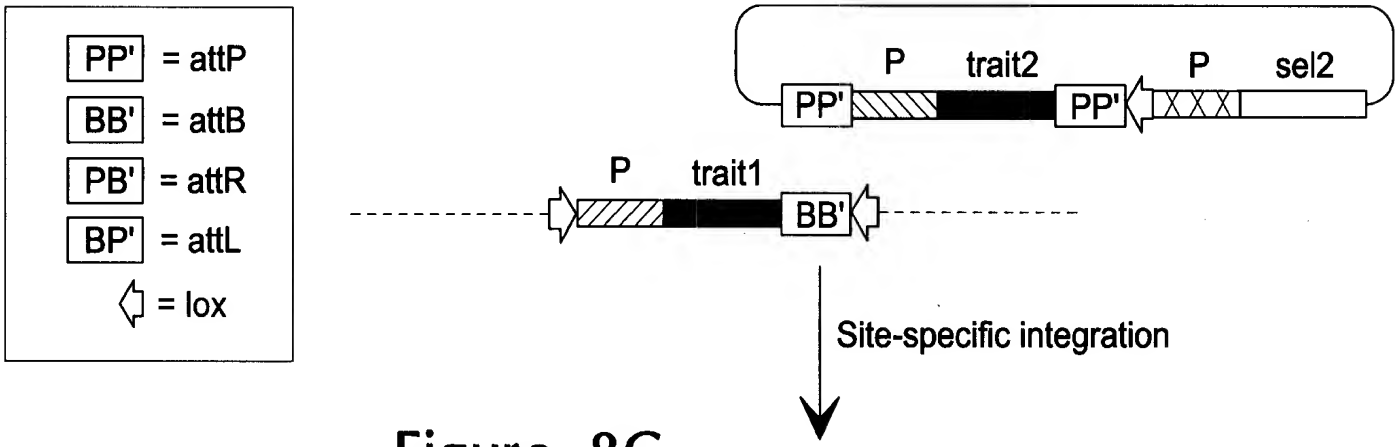


Figure 8C

General strategy to stack genes, part1
Use of directly oriented sites

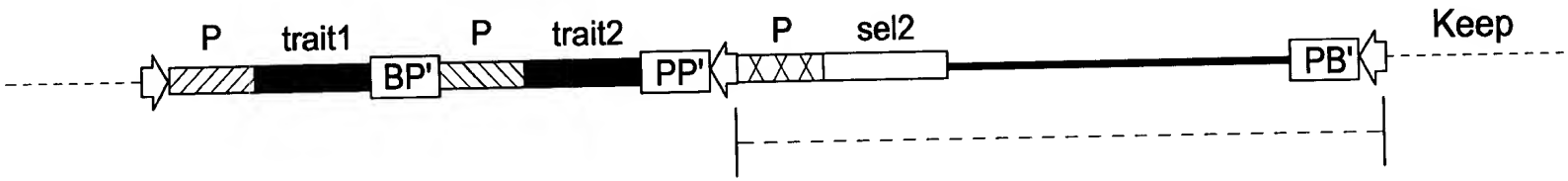
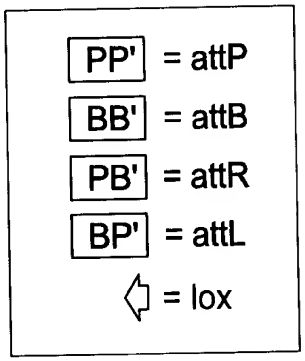


Figure 8D



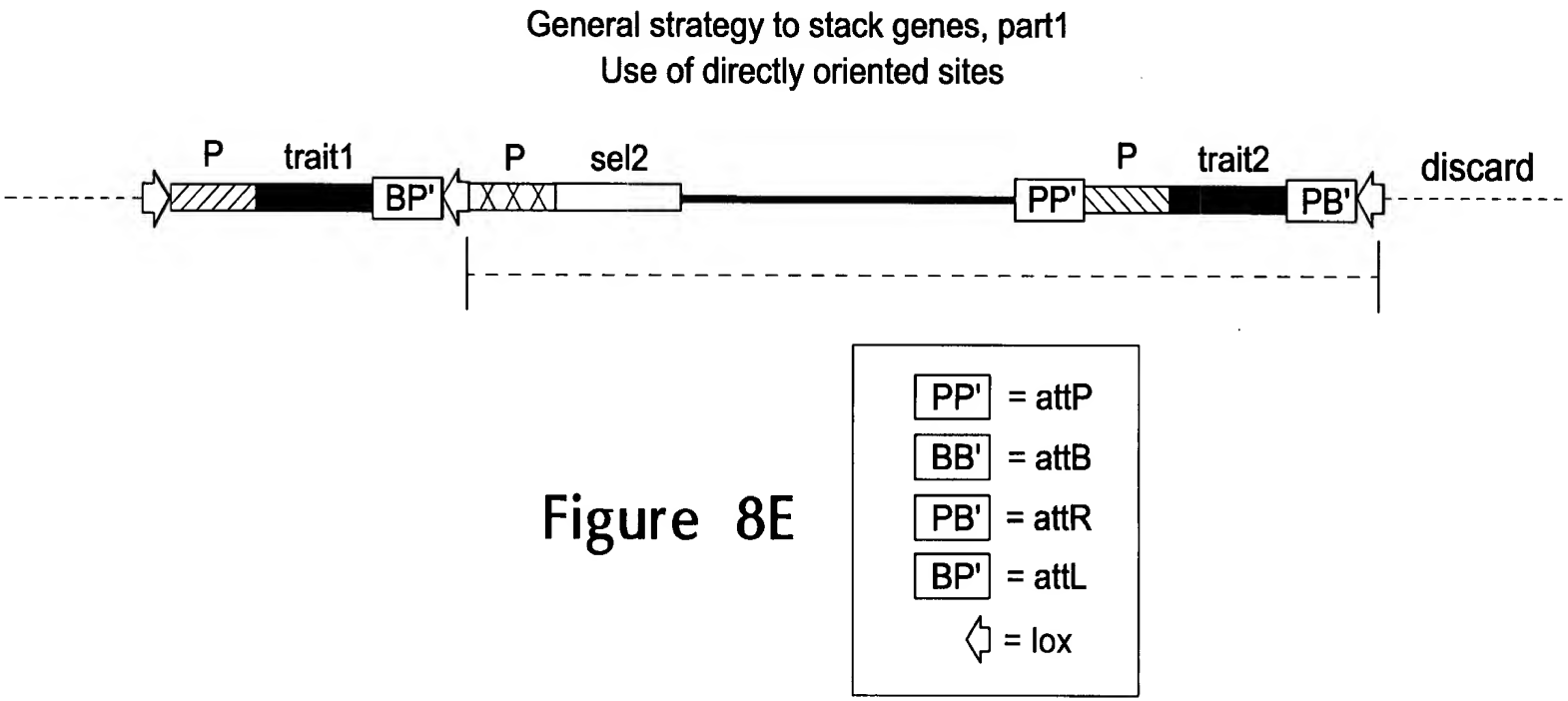

General strategy to stack genes, part1
Use of directly oriented sites
P
trait1
BP'
P
sel2
P
trait2
discard
PP'
PB'
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 8E

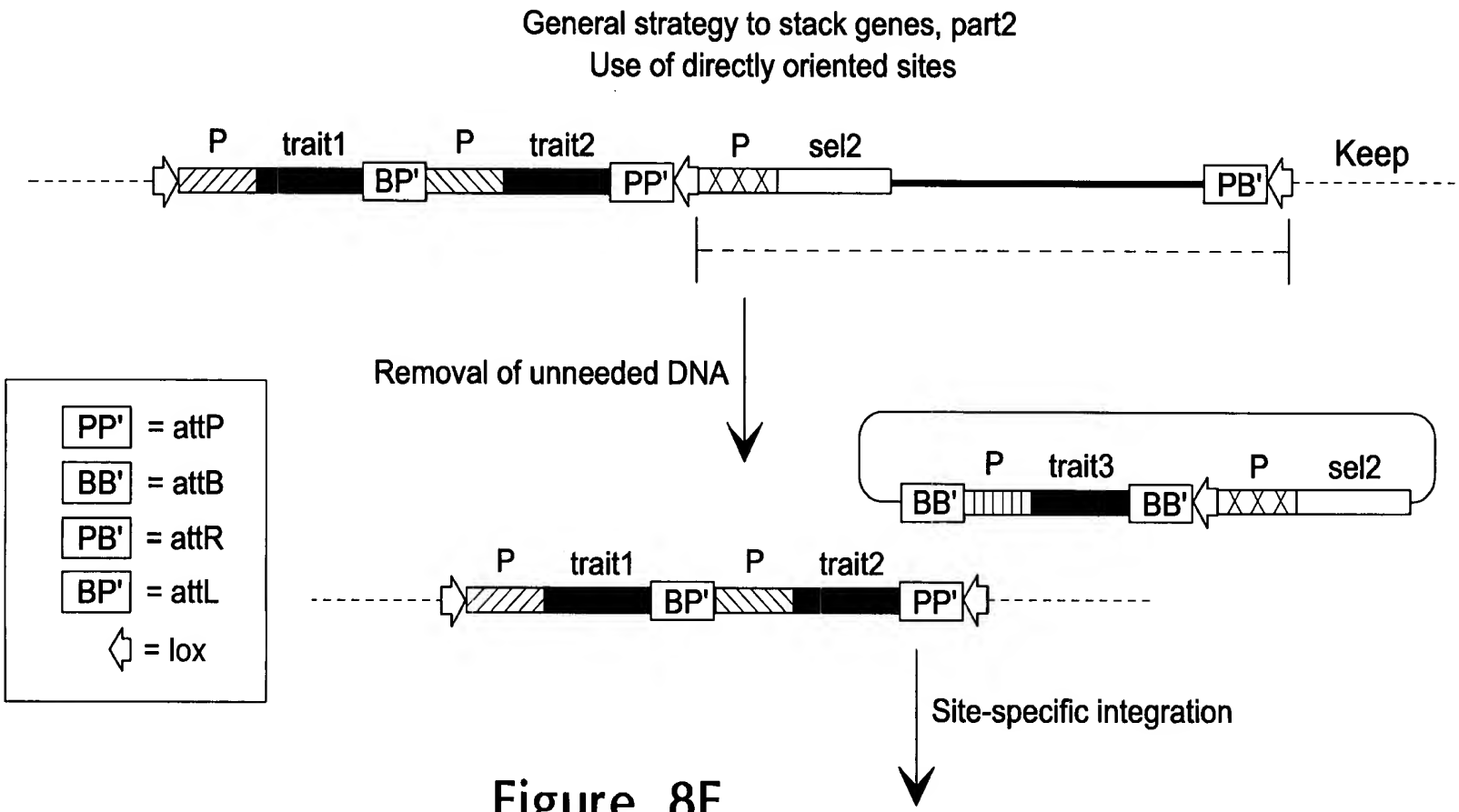

General strategy to stack genes, part2
Use of directly oriented sites
P
trait1
BP'
P
trait2
PP'
P
sel2
PB'
Keep
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox
P
trait3
BB'
P
sel2
BB'
P
trait1
BP'
P
trait2
PP'
Site-specific integration

Figure 8F

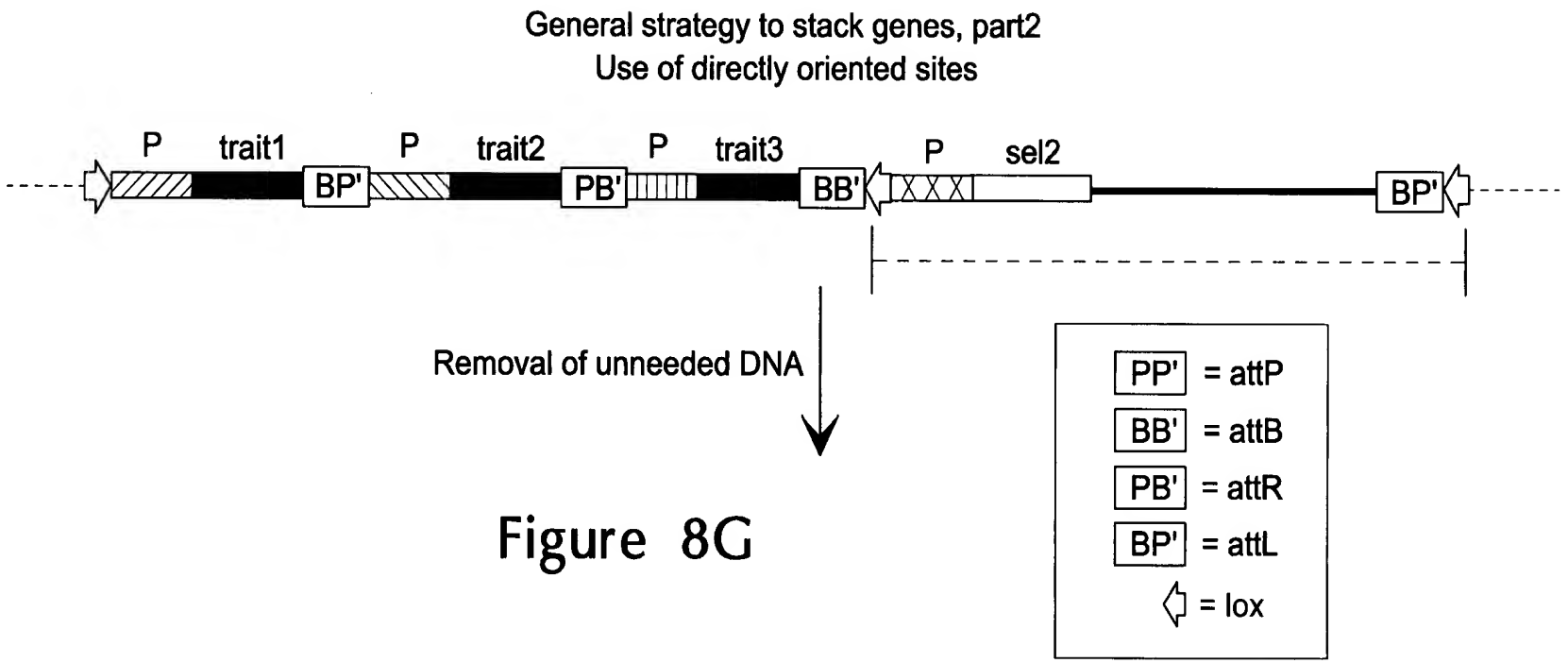

General strategy to stack genes, part2
Use of directly oriented sites
P
trait1
BP'
P
trait2
PB'
P
trait3
BB'
P
sel2
BP'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 8G

General strategy to stack genes, part2
Use of directly oriented sites

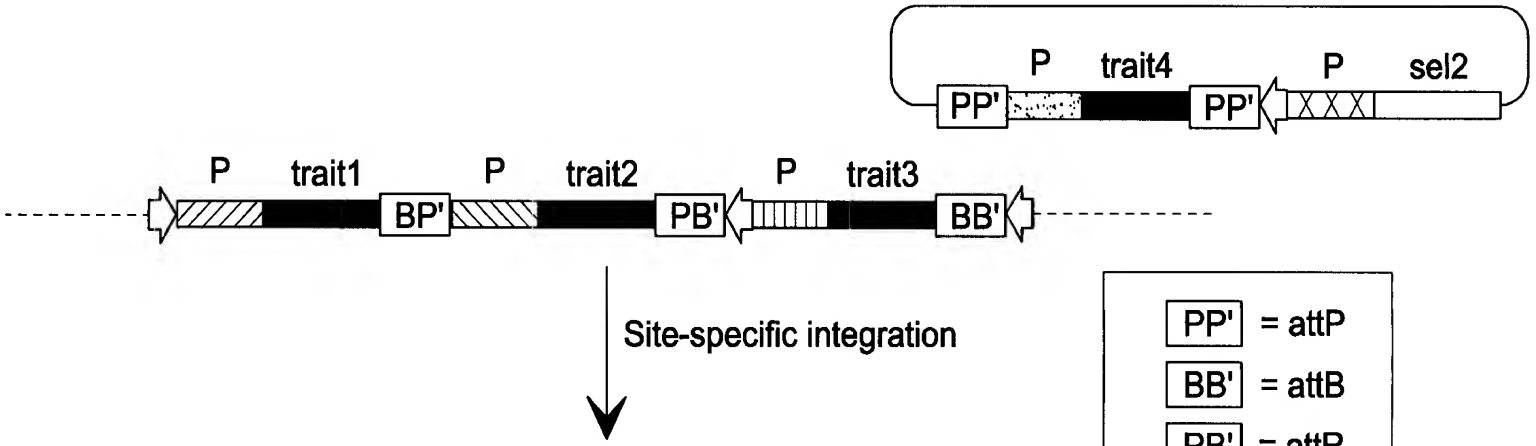


Figure 8H

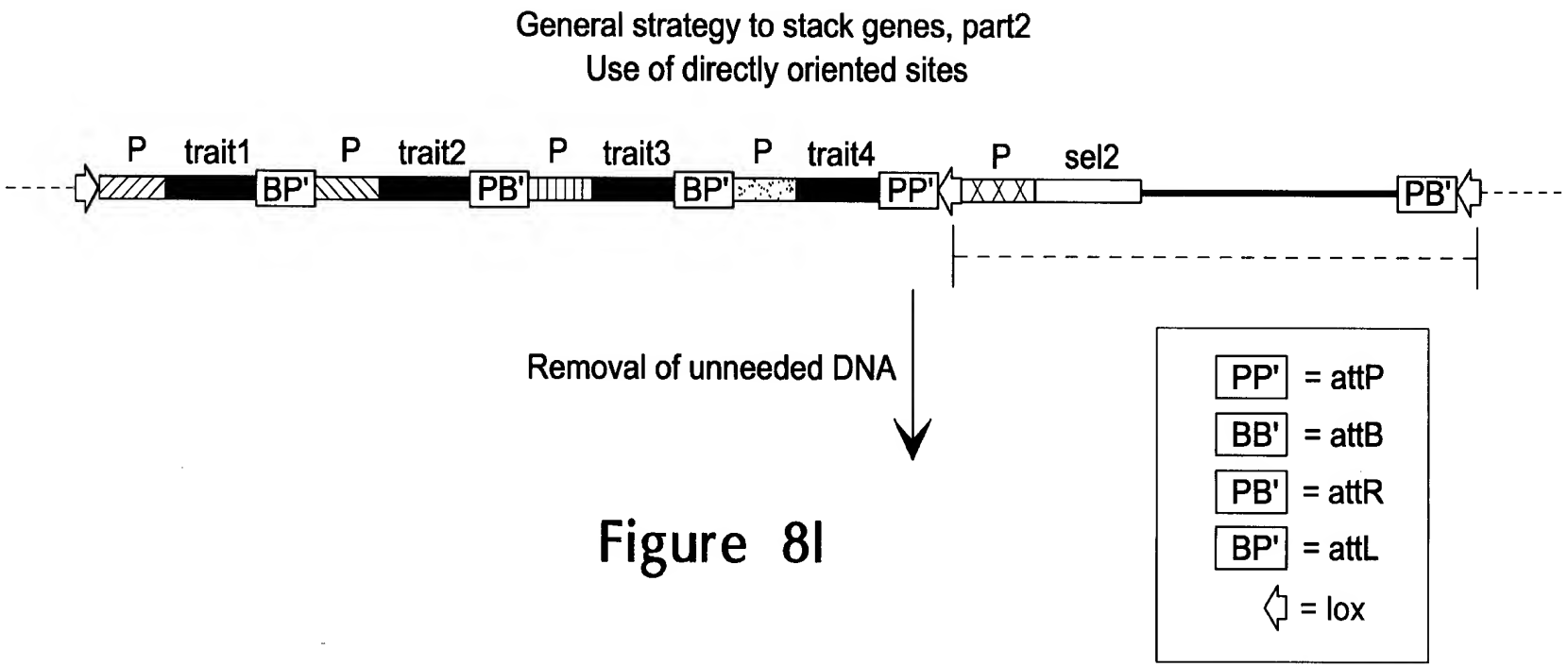

General strategy to stack genes, part2
Use of directly oriented sites
P
trait1
BP'
P
trait2
PB'
P
trait3
BP'
P
trait4
PP'
P
sel2
PB'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 8l

General strategy to stack genes, part2
Use of directly oriented sites

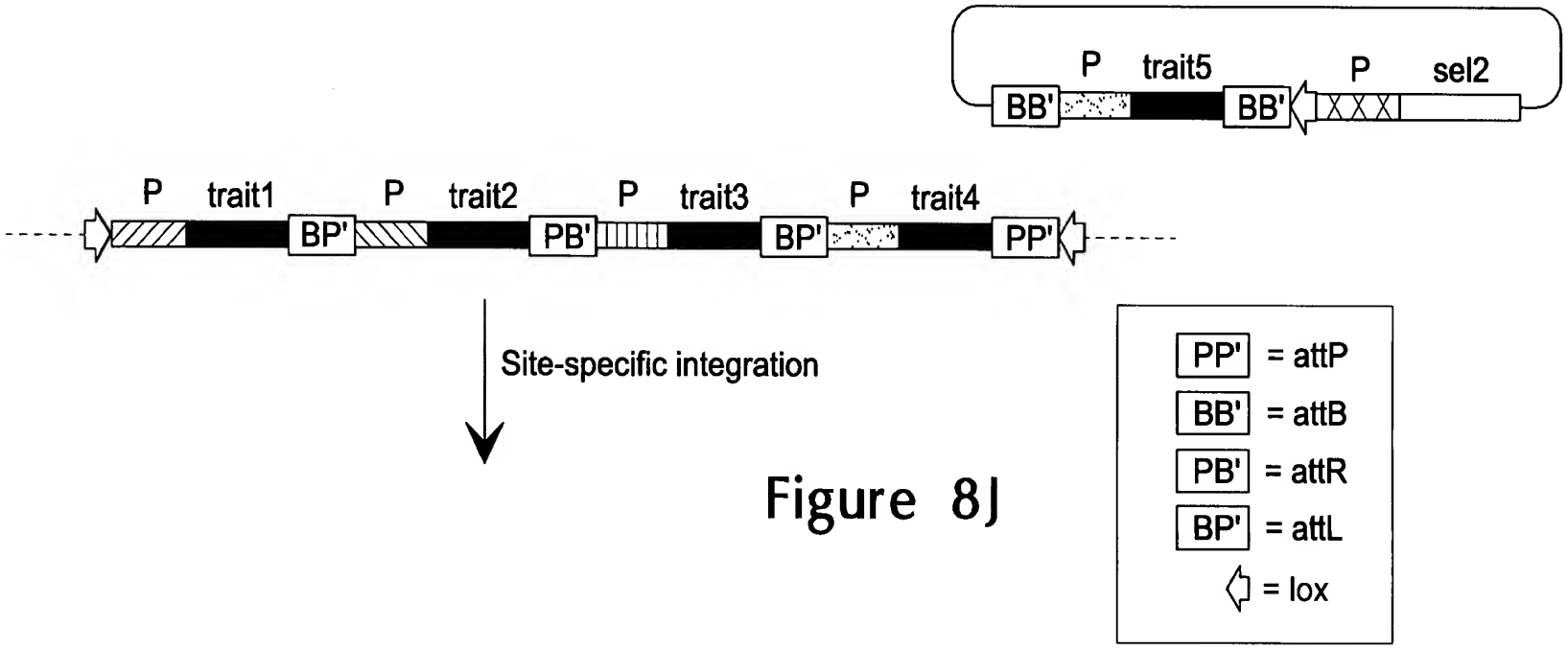


Figure 8J

General strategy to stack genes, part1
Use of inverted sites

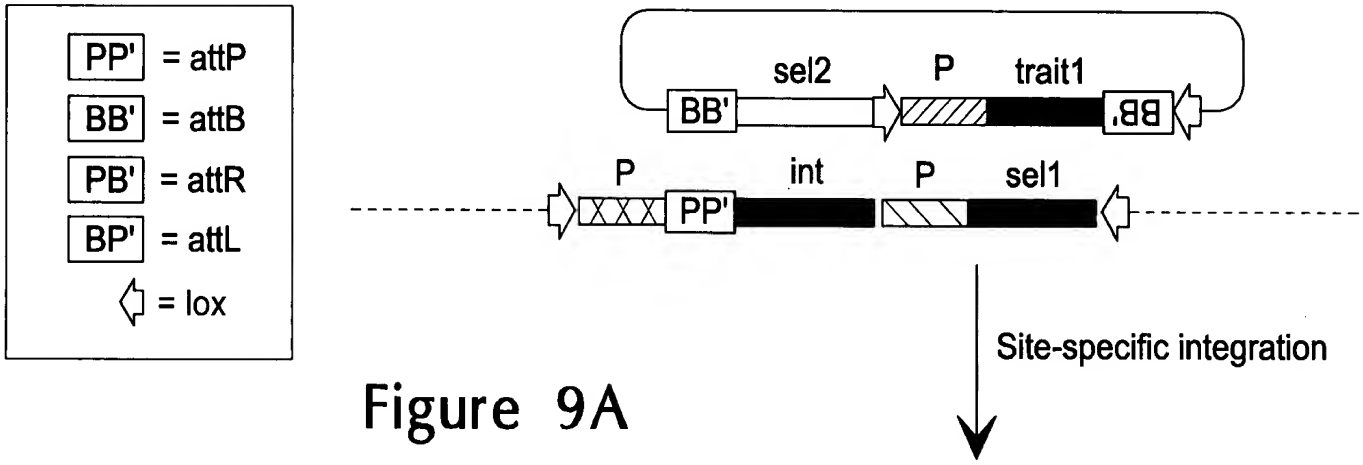


Figure 9A

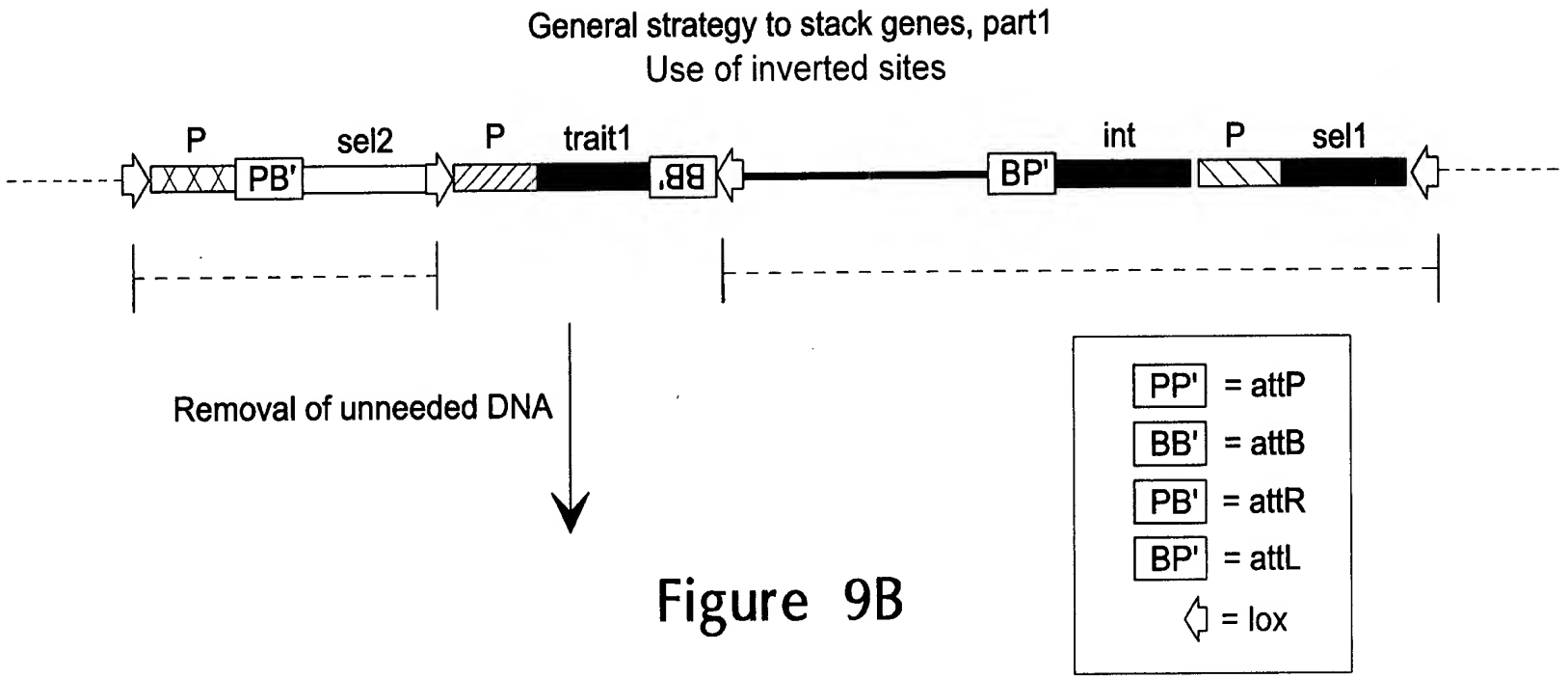

General strategy to stack genes, part1
Use of inverted sites
P
sel2
P
trait1
int
P
sel1
PB'
BB'
BP'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 9B

General strategy to stack genes, part1
Use of inverted sites

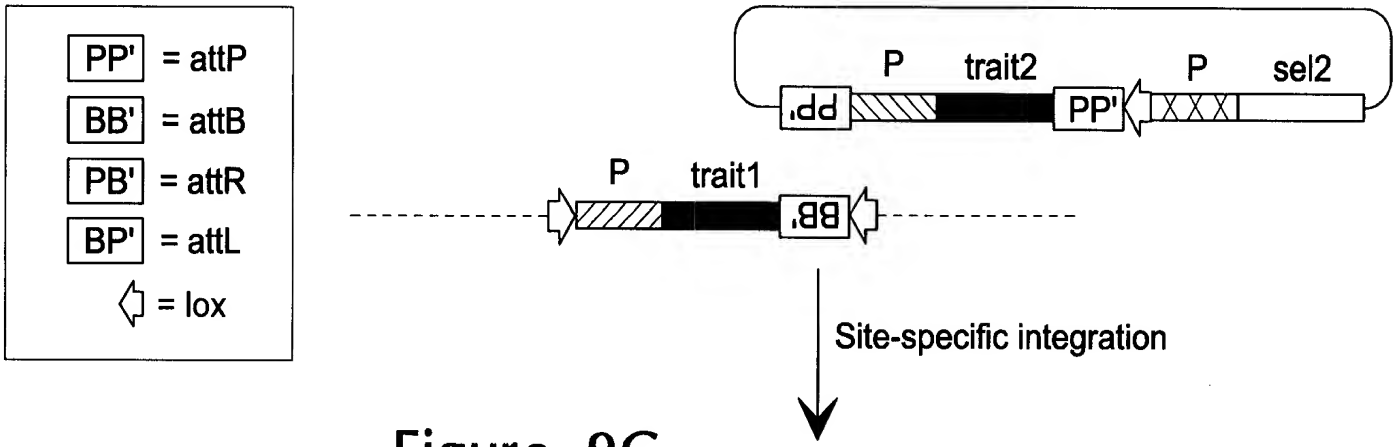


Figure 9C

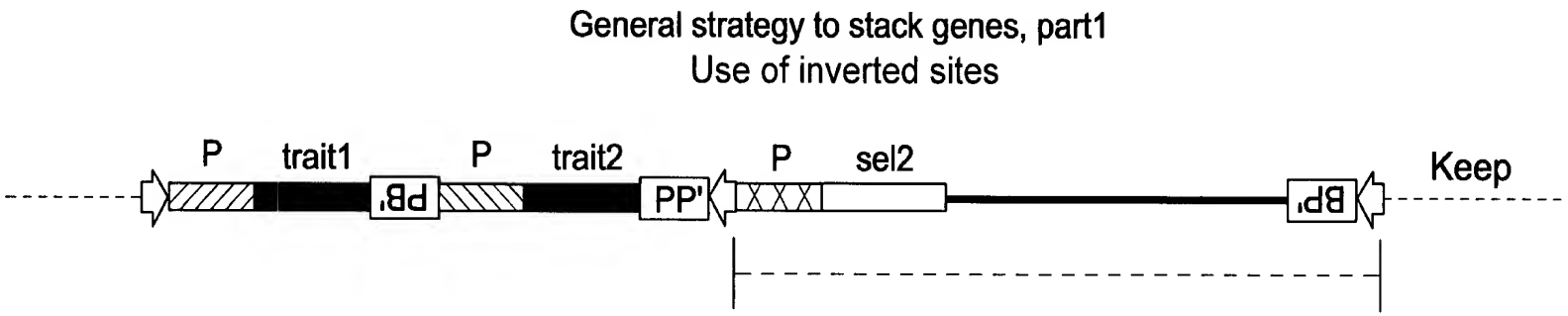

General strategy to stack genes, part1
Use of inverted sites
P
trait1
P
trait2
P
sel2
Keep
PB'
PP'
BP'
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 9D

General strategy to stack genes, part1
Use of inverted sites

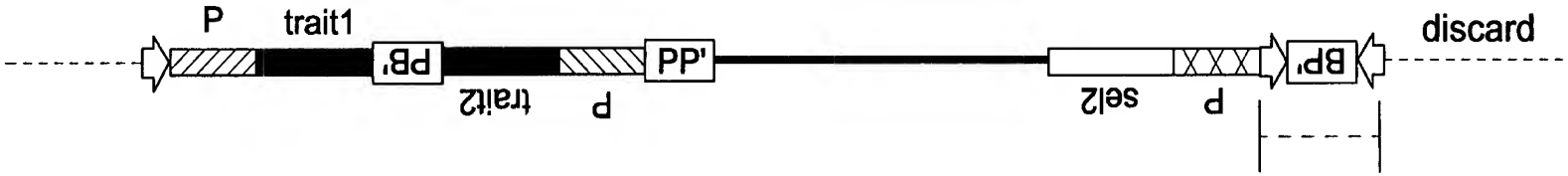
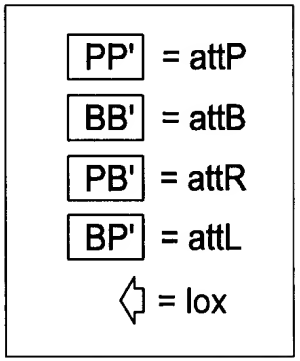


Figure 9E



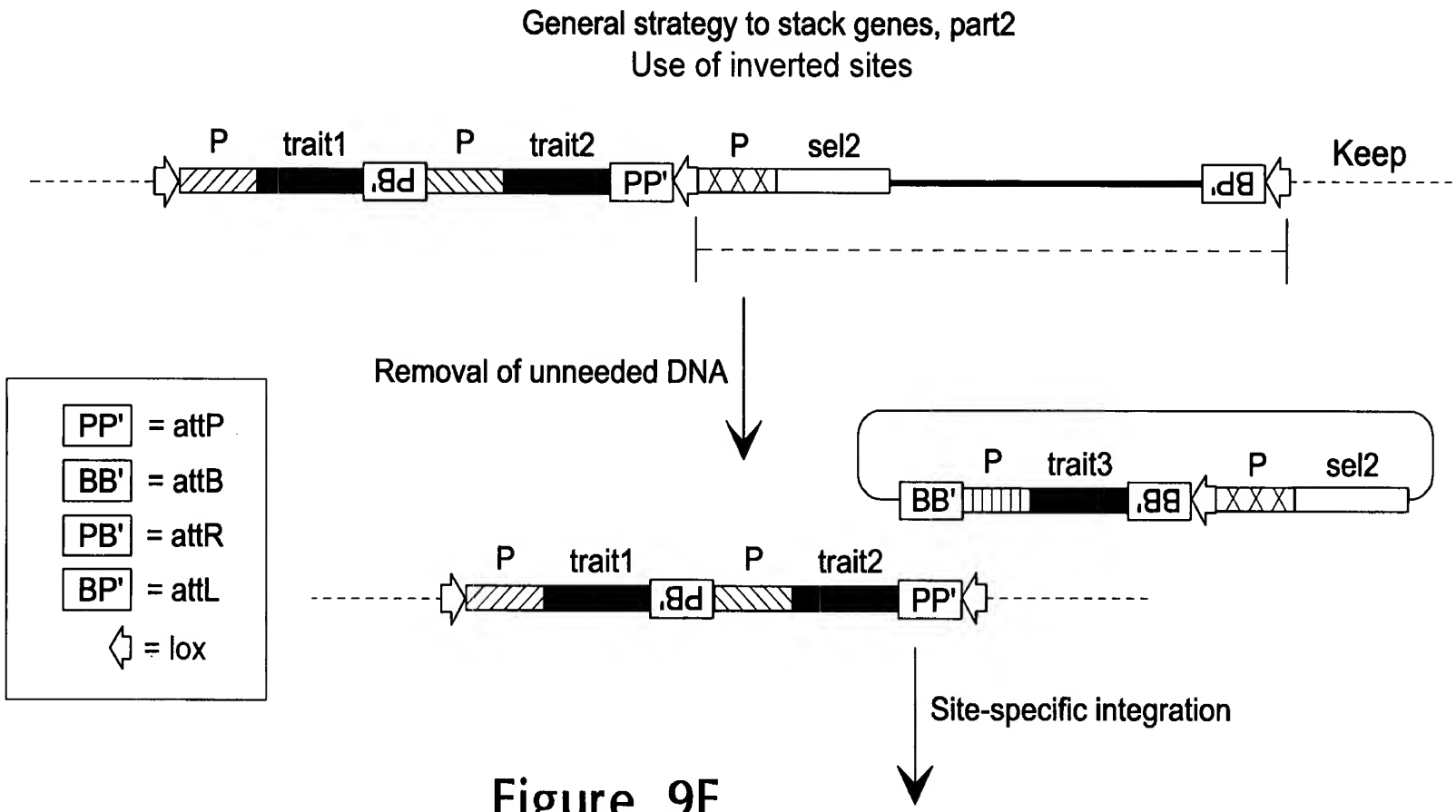

General strategy to stack genes, part2
Use of inverted sites
P
trait1
P
trait2
P
sel2
Keep
PB'
PP'
BP'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox
P
trait3
P
sel2
BB'
BB'
P
trait1
P
trait2
PB'
PP'
Site-specific integration

Figure 9F

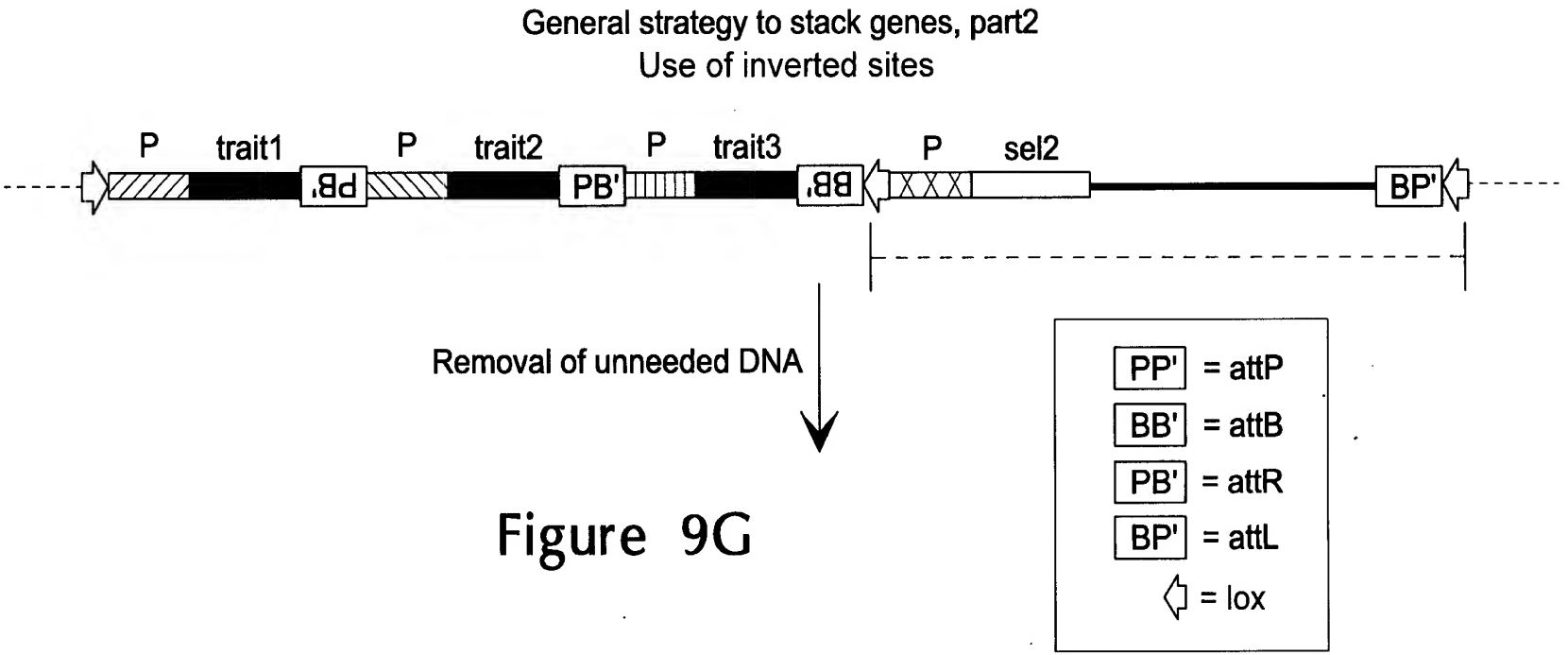

General strategy to stack genes, part2
Use of inverted sites
P
trait1
P
trait2
P
trait3
P
sel2
PB'
BB'
BP'
Removal of unneeded DNA
PP' = attP
BB' = attB
PB' = attR
BP' = attL
= lox

Figure 9G

General strategy to stack genes, part2
Use of inverted sites

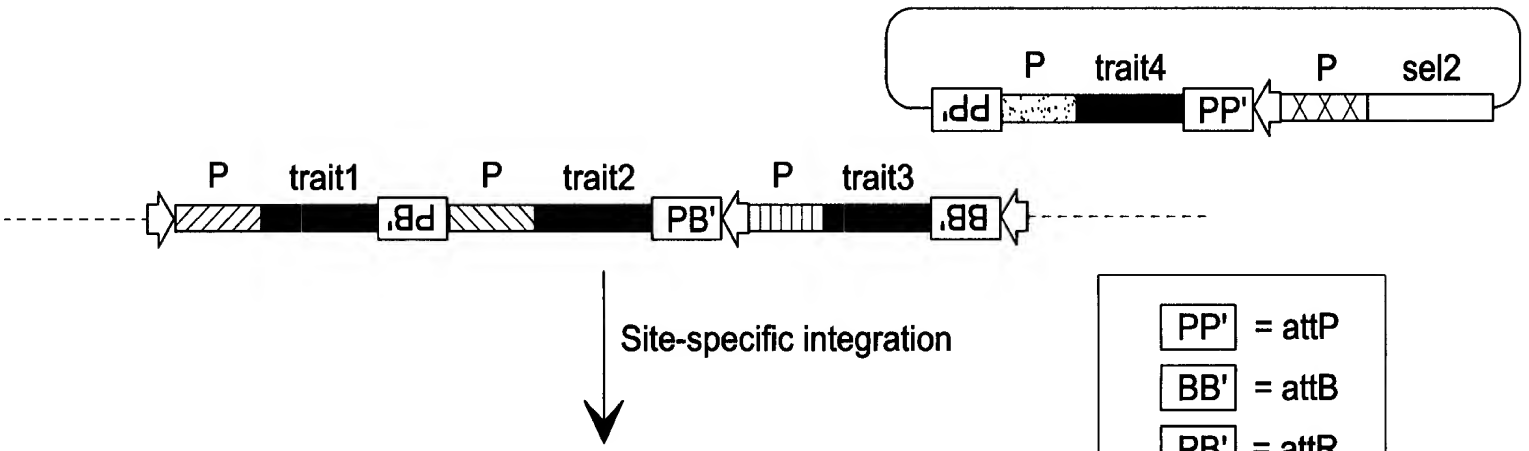
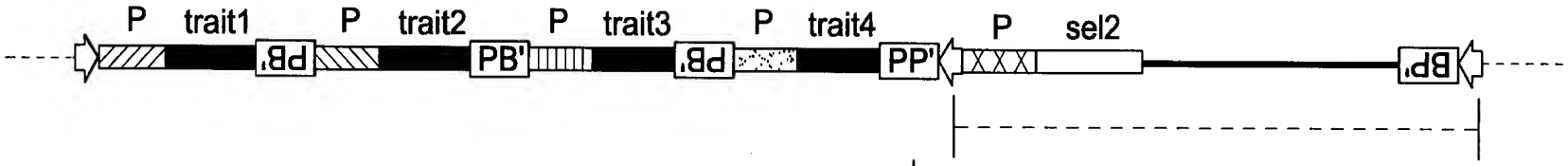


Figure 9H

General strategy to stack genes, part2
Use of inverted sites



Removal of unneeded DNA

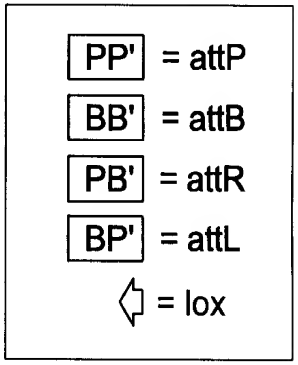


Figure 9l

General strategy to stack genes, part2
Use of inverted sites

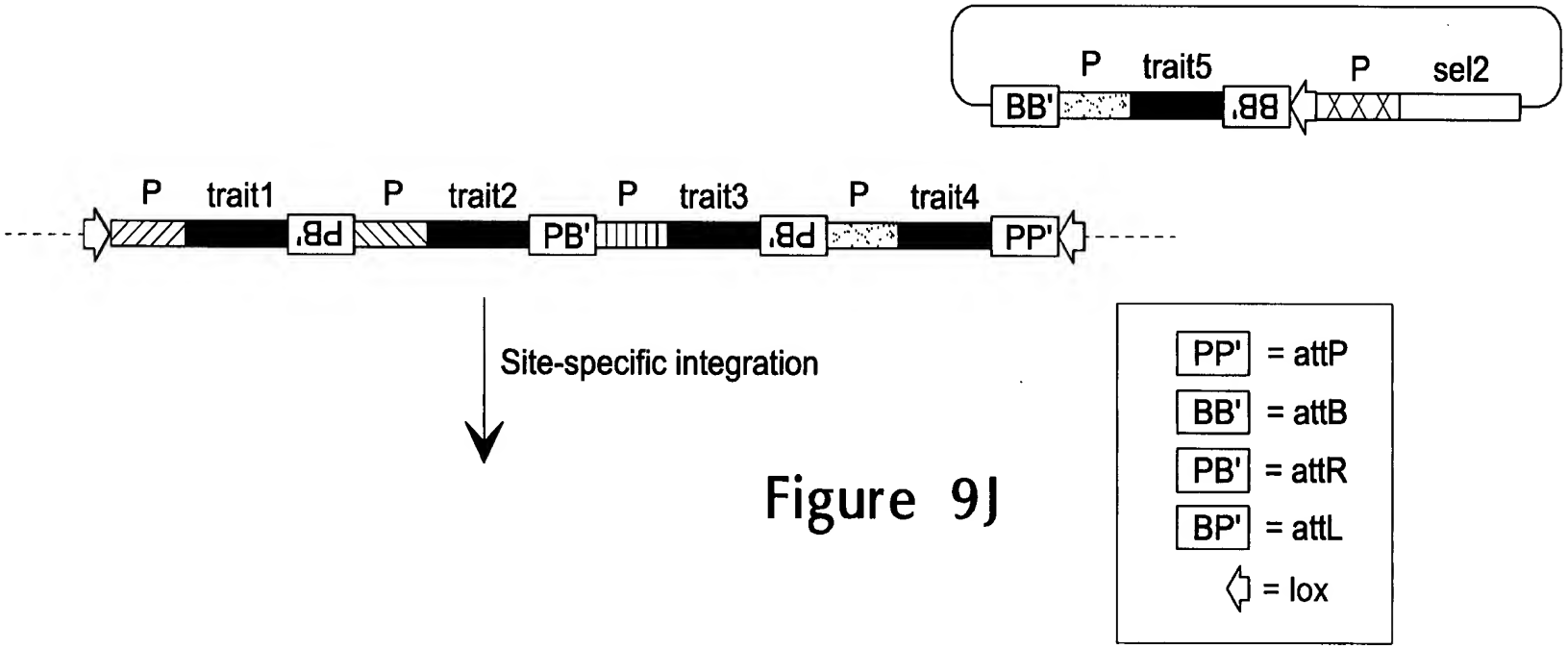


Figure 9J

Gene replacement in the host genome with directly oriented dual sites

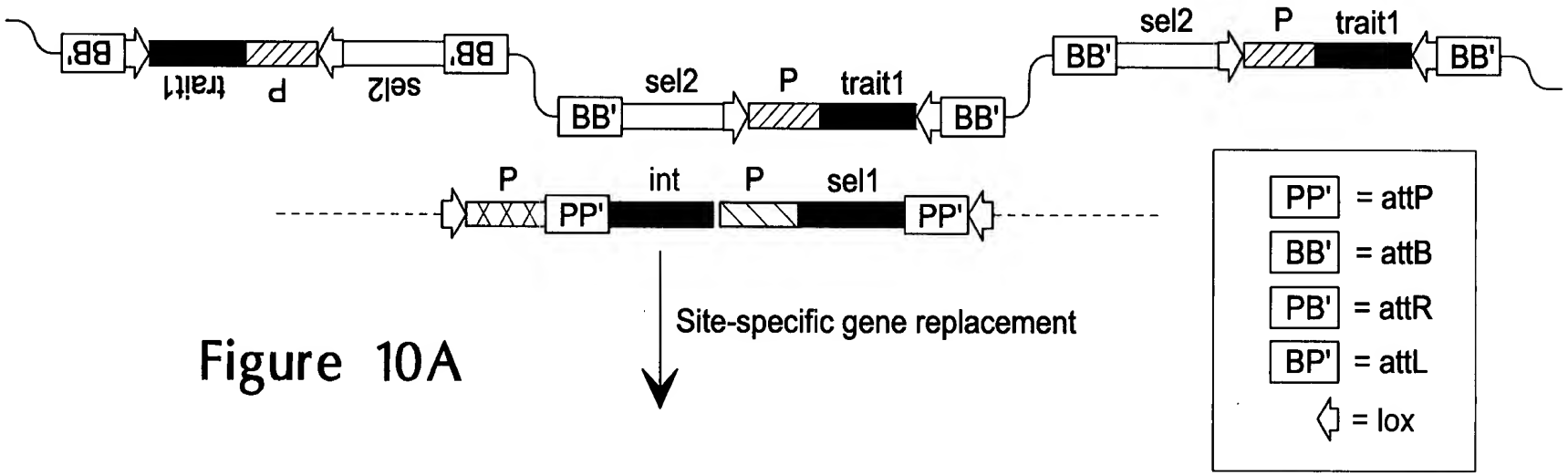


Figure 10A

Gene replacement in the host genome with directly oriented dual sites

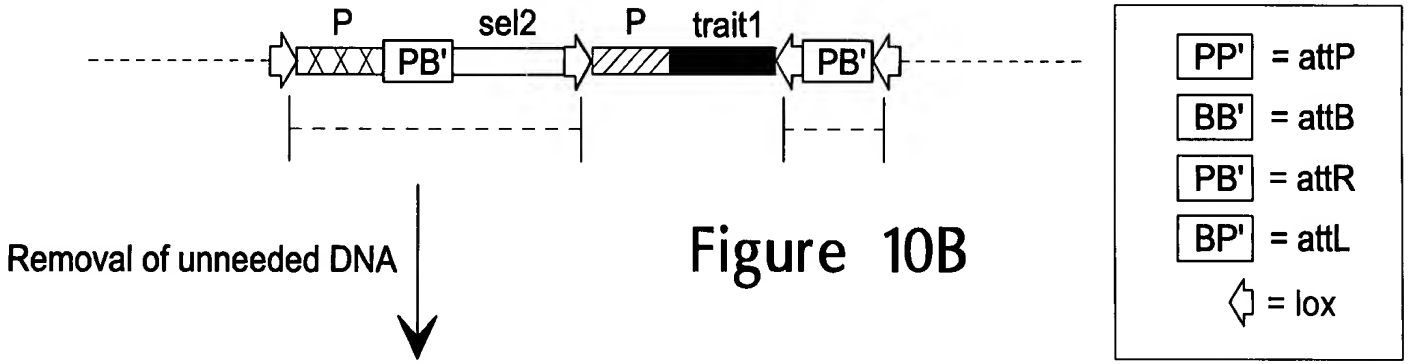


Figure 10B

Gene replacement in the host genome with directly oriented dual sites

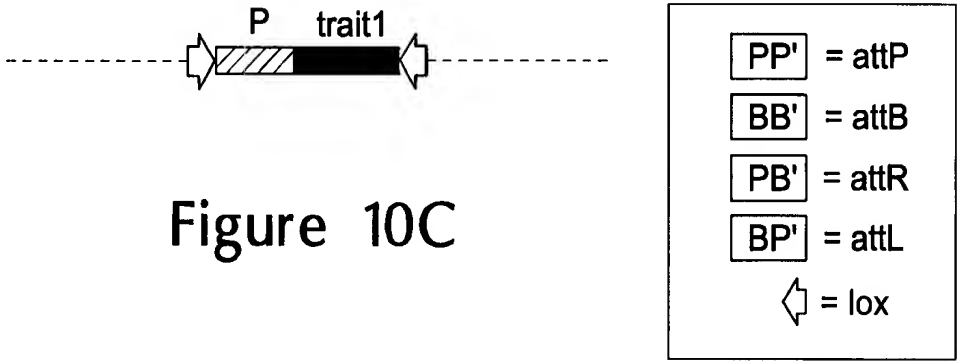


Figure 10C

Gene replacement in the host genome with inverted dual sites

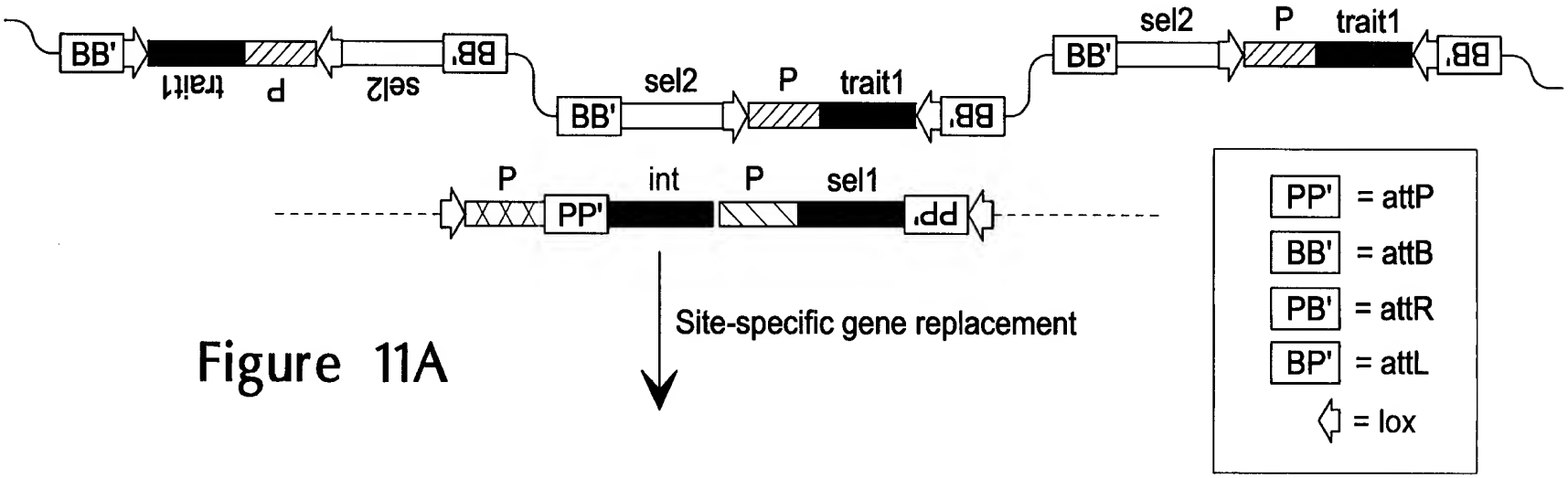


Figure 11A

Gene replacement in the host genome with inverted dual sites

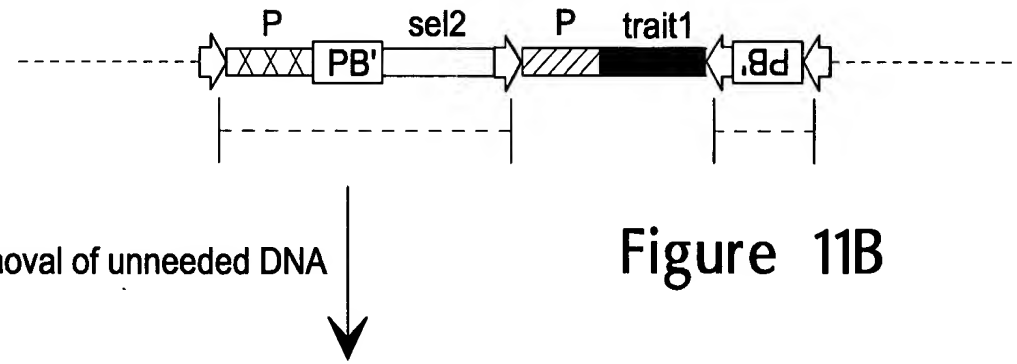


Figure 11B

Gene replacement in the host genome with inverted dual sites

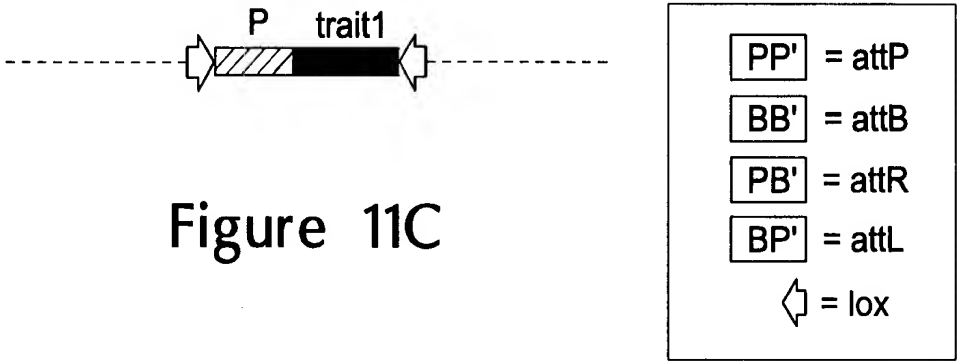


Figure 11C

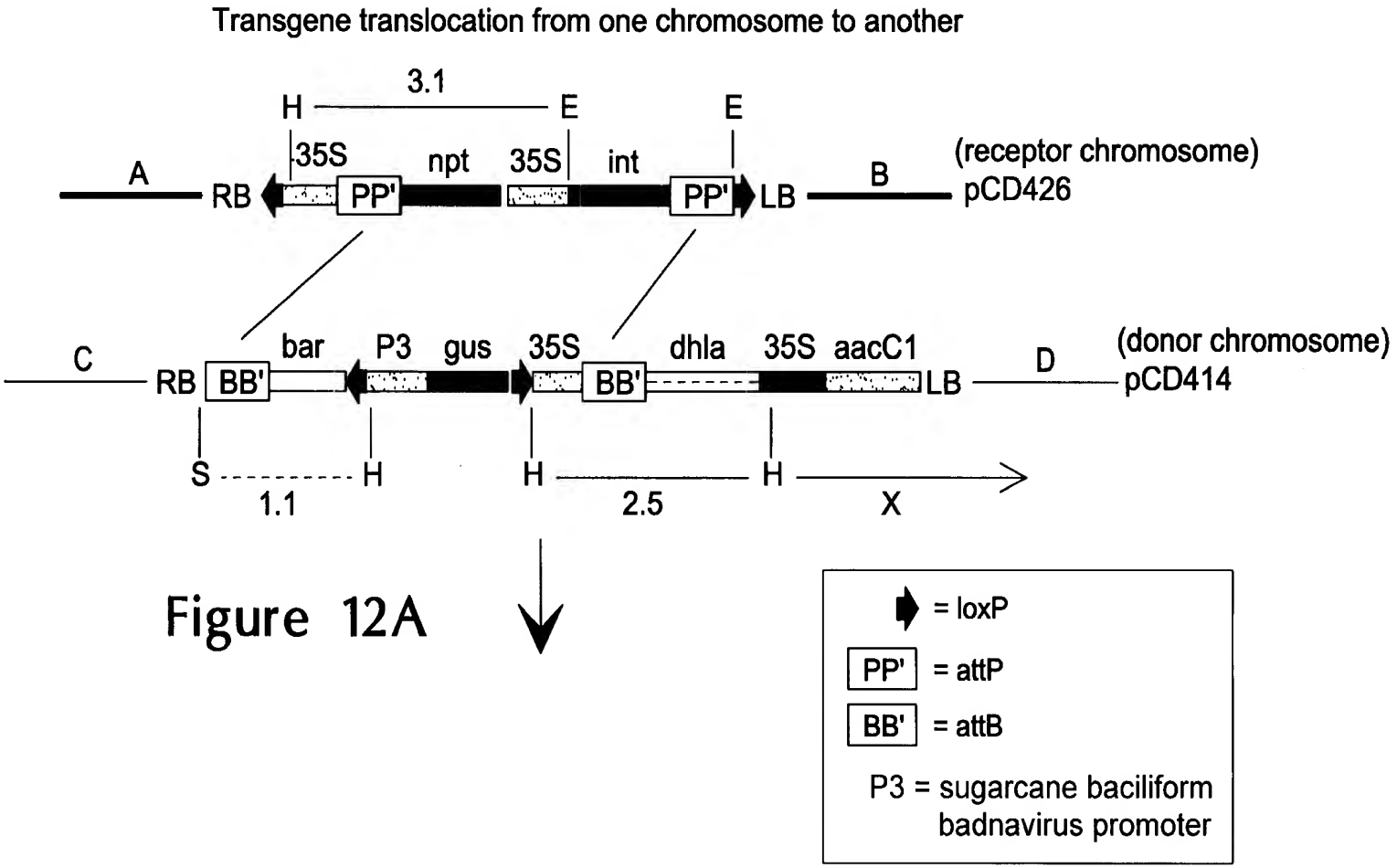

Transgene translocation from one chromosome to another
3.1
H
E
E
35S
npt
35S
int
A
RB
PP'
PP'
LB
B
(receptor chromosome)
pCD426
C
RB
BB'
bar
P3
gus
35S
BB'
dhla
35S
aacC1
LB
D
(donor chromosome)
pCD414
S
H
H
H
1.1
2.5
X
= loxP
PP' = attP
BB' = attB
P3 = sugarcane baciliform badnavirus promoter

Figure 12A

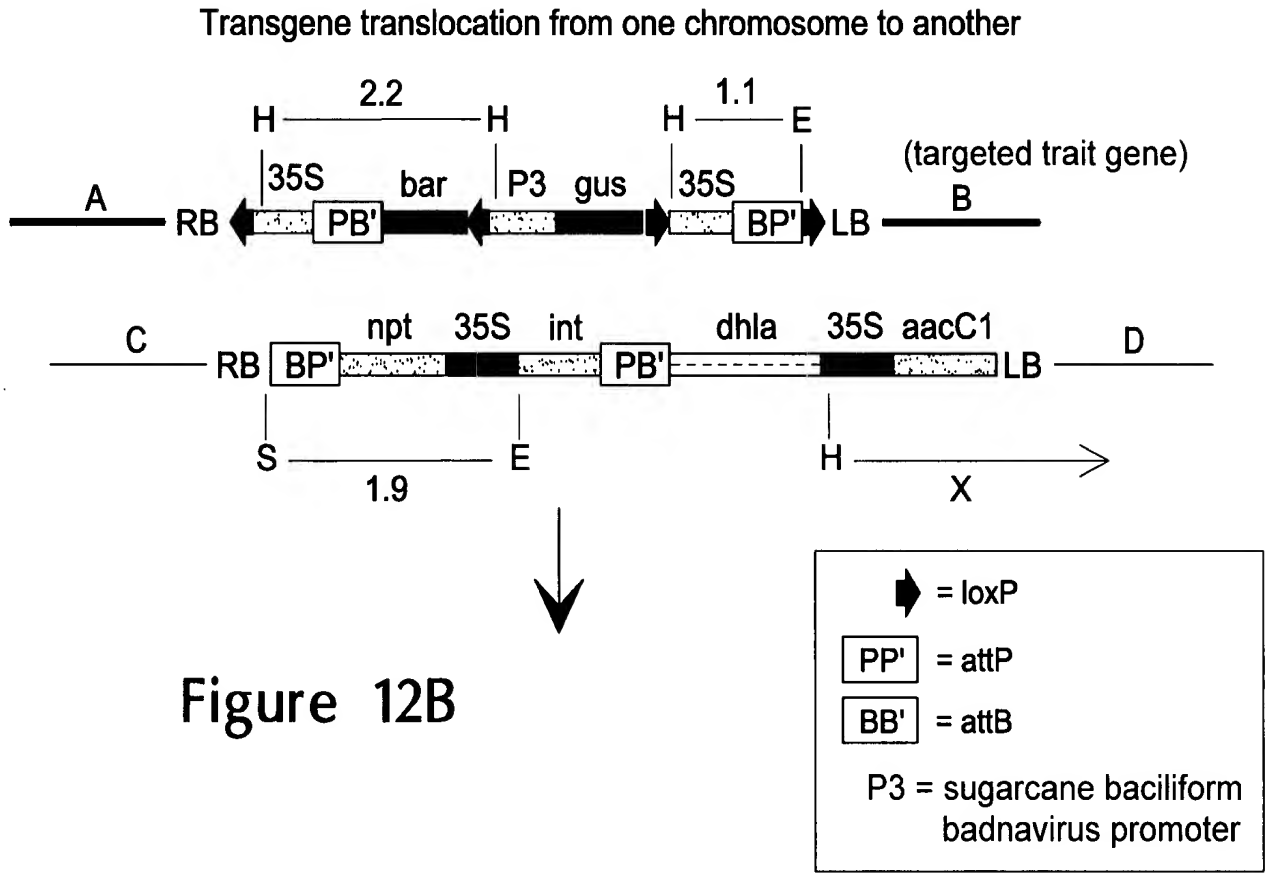

Transgene translocation from one chromosome to another
2.2
1.1
H
H
H
E
35S
bar
P3
gus
35S
(targeted trait gene)
A
RB
PB'
BP'
LB
B
C
npt
35S
int
dhla
35S
aacC1
D
RB
BP'
PB'
LB
S
E
H
1.9
X
= loxP
PP' = attP
BB' = attB
P3 = sugarcane baciliform badnavirus promoter

Figure 12B

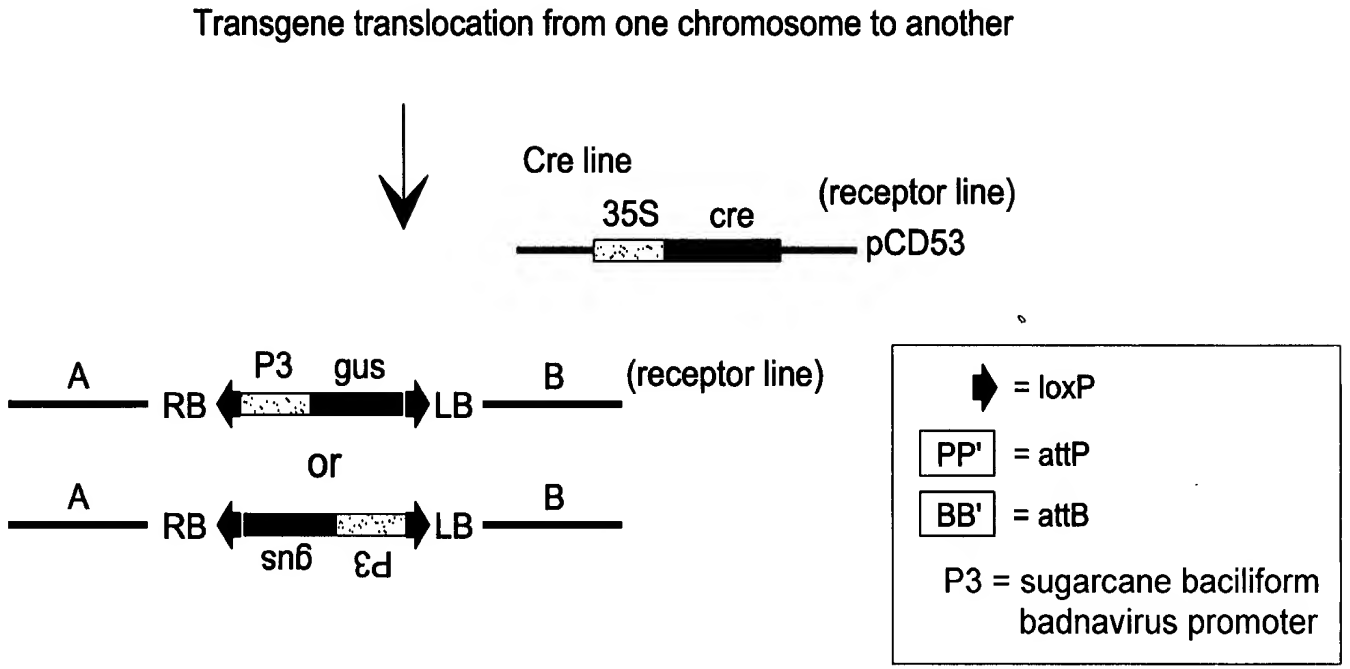

Transgene translocation from one chromosome to another
Cre line
35S
cre
(receptor line)
pCD53
A
RB
P3
gus
LB
B
(receptor line)
or
A
RB
gus
P3
LB
B
= loxP
PP'
= attP
BB'
= attB
P3 = sugarcane baciliform badnavirus promoter

Figure 12C

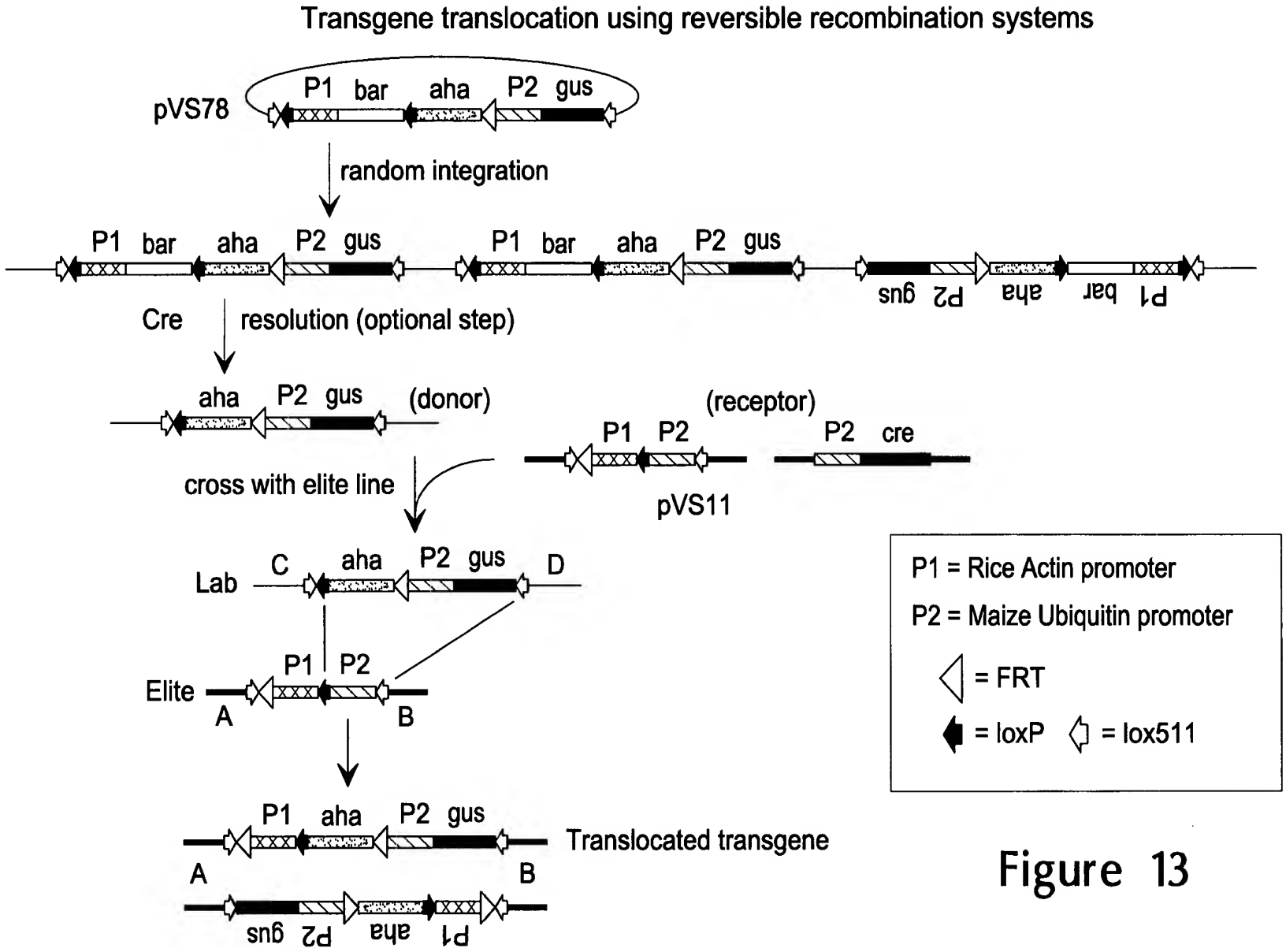

Transgene translocation using reversible recombination systems
pVS78
P1 bar aha P2 gus
random integration
P1 bar aha P2 gus
P1 bar aha P2 gus
Cre
resolution (optional step)
aha P2 gus (donor)
(receptor)
P1 P2
P2 cre
pVS11
cross with elite line
Lab
C aha P2 gus D
Elite
P1 P2
A
B
P1 aha P2 gus
Translocated transgene
A
B
P1 aha P2 gus
P1 = Rice Actin promoter
P2 = Maize Ubiquitin promoter
= FRT
= loxP
= lox511

Figure 13